



HEALTH HOLDING

HAFER ALBATIN HEALTH
CLUSTER
MATERNITY AND
CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Chemistry)		
Document:	Internal Policy and Procedure		
Title:	Analysis of Direct Bilirubin Level		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

- 1.1 The purpose of this policy and procedure is to provide all information related to the analysis of Direct Bilirubin level in blood (serum/plasma) on DimensionEXL200 ,Synchron DXC700 and Atelica CI machines.

2. DEFINITONS:

- 2.1 Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes.

3. POLICY:

- 3.1 This policy provides instructions for performing the quantitative determination of Direct Bilirubin in human serum or plasma on DimensionEXL200 ,Synchron DXC700 and Atelica CI machines.
- 3.2 Bilirubin is formed in the reticuloendothelial system during the degeneration of aged erythrocytes. The haem-portion from haemoglobin and from other haem-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronicacid for solubilisation and subsequent transport through the bile duct and elimination via the digestive tract.
- 3.3 Bilirubin elevated in liver and biliary diseases including: hepatitis, gall stones, biliary cirrhosis, hepatic malignancy, cholestatic drug reactions. It is increased physiologically during the first few days following birth due to hepatic dysfunction, biliary obstruction or excessive red cell turnover.

4. PROCEDURE:

4.1 Specimen:

- 4.1.1 Type:
 - 4.1.1.1 Serum, or plasma
- 4.1.2 Tube Type:
 - 4.1.2.1 Gel tube, Plain tube, Li-Heparin
- 4.1.3 Amount Required:
 - 4.1.3.1 2.0 to 3.0 ml.
- 4.1.4 Delivery Arrangements:
 - 4.1.4.1 Sample to be delivered to the lab as soon as possible.
- 4.1.5 If the sample is serum should be ensuring complete clot formation before centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- 4.1.6 Temperature Restrictions:
 - 4.1.6.1 At room temperature.
- 4.1.7 Unacceptable Specimen:
 - 4.1.7.1 See sample rejection criteria policy.
- 4.1.8 Specimen Retention:
 - 4.1.8.1 Period of retention: Up to one week after separation of the sample

- 4.1.8.2 Storage condition: Store at 2-8 °T
- 4.1.9 Safety Precaution:
 - 4.1.9.1 Treat all samples material as infectious and handled in accordance with the OHS standard on blood borne pathogens.
- 4.2 **Principle:**
 - 4.2.1 Conjugated bilirubin + diazotized sulfanilic acid -----> Red chromophore
 - 4.2.2 The colour intensity is directly proportional to the Direct Bilirubin concentration in the sample and is measured photometrically at 540 nm.
- 4.3 **Method:**
 - 4.3.1 See policy of loading sample on machine (Ref: Operative Manuals' of DimensionEXL200 ,Synchron DXC700 and Atelica CI).
- 4.4 **Calculation:**
 - 4.4.1 Instrument system automatically calculates the Analytic activity and gives results in the form of printout.
- 4.5 **Format:**
 - 4.5.1 Numeric
- 4.6 **Status:**
 - 4.6.1 Stat and Routine
- 4.7 **Reference ranges:**
 - 4.7.1 Serum/plasma 0.0 - 3.42 umol/L
- 4.8 **Dilution information:**
 - 4.8.1 Specimens with values exceeding the linearity range are flagged and may be diluted with automatic dilution either automated or manual dilution. Manual Dilution should be performed as follows:
 - 4.8.1.1 Use saline (0.85% to 0.90%) to dilute the sample
 - 4.8.1.2 The operator must enter the dilution factor in the patient order screen. The system dilution factor to automatically correct the concentration by multiplying the result by factor.
 - 4.8.1.3 If the operator does not enter the dilution factor, the result must be multiplied appropriate dilution factor before reporting the result.
 - 4.8.1.4 If a diluted sample result generates a Linear Low (LL) result error code. Do result.
 - 4.8.1.5 Prepare an appropriate dilution/concentration and rerun.
- 4.9 **Linearity:**
 - 4.9.1 Method is linear up to 273.6umol/L
- 4.10 **Limit of Detection:**
 - 4.10.1 The Limit of Detection is 0.855 umol/L

5. MATERIALS AND EQUIPMENT:

5.1 Reagents:

5.1.1 Direct Billrubin flex is ready to use cassettes with the following ingredients:

Reactive Ingredients	Ingredient Concentration
(1-4 wells)	
Empty	
Liquid (5 well)	
Sodium Nitrite	72.5 mM
Liquid (6 well)	
Hydrochloric acid	500 mM
Liquid (7-8 wells)	
Sulfanilic acid	25.89 mM
Hydrochloric acid	132 mM

5.1.1.1 Reagent Preparation:

- 5.1.1.1.1 Mixing and diluting are automatically performed by the Dimension system.
- 5.1.1.1.2 Estimated test per cassette, 40 tests
- 5.1.1.1.3 Analytical Range: Serum/plasma 0.855—273 umol/L

- 5.1.2 Reagents retention:
 - 5.1.2.1 The unopened reagents are stable until the expiration date when stored at 2-8° C. Reagent stability is 30 days if the reagent is unopened and for 2 days if the reagent is opened well for wells 1-4 (once the diazotized sulfanilic acid has been prepared) and 30 days for wells 5-8.
- 5.2 **Calibration:**
 - 5.2.1 Calibration is stable approximately 30 days and required with each change in reagent lot number. Verify calibration curve with at least two levels of controls according to the established Quality Control requirements for your laboratory. Calibration must be done when:
 - 5.2.1.1 A complete change of reagents that affects the range used to report patient results or QC value.
 - 5.2.1.2 A reagent kit with new lot number is used.
 - 5.2.1.3 A new assay file that requires a calibration is installed.
 - 5.2.1.4 QC fails to meet the established criteria.
 - 5.2.1.5 After major maintenance or service.
 - 5.2.1.6 When recommended by the manufacturer.
 - 5.2.1.7 Documentation accompanying a new version of an existing file states calibration is required.
 - 5.2.1.8 At least every 6 months.
 - 5.2.2 Calibrator retention:
 - 5.2.2.1 At 2-8 C for 24 h. Instability or deterioration should be suspected if there are visible signs of leakage, extreme turbidity microbial growth or if calibration does not meet the appropriate package insert and/or instrument operation manual criteria.
 - 5.2.3 Calibration Procedure:
 - 5.2.3.1 Verify that the correct calibrator values have been entered into the calibration file. For details refer to Operator Guide of DimensionEXL200.
 - 5.2.3.2 Allow calibrator to come to room temperature.
 - 5.2.3.3 Mix bottle 10 times by inversion.
 - 5.2.3.4 Open the bottle, place a minimum of 300 ul of each level in separate sample cup, and place on the assigned positions.
 - 5.2.3.5 Cap the bottle tightly and store at 2-8°C. Immediately after use.
 - 5.2.3.6 Perform calibration as indicated in Operator Guide of DimensionEXL200 ,Synchron DXC700 and Atelica CI
 - 5.2.4 Calibration Expected Values:
 - 5.2.4.1 Refer to TBI/DBI calibrator for Dimension
 - 5.2.4.2 Refer to DimensionEXL200 ,Synchron DXC700 and Atelica CI calibrator leaflet
- 5.3 **Quality control:**
 - 5.3.1 Normal and pathological control. one time in 24 hours. If more frequent control monitoring is required, the established quality control procedure is followed. If quality control results do not fall within an acceptable range defined by laboratory, patient be affected and corrective action should be taken.
 - 5.3.2 Quality Control retention:
 - 5.3.2.1 Unopened control vial is stable up to expiry date printed on the label when stored at cold room.
 - 5.3.2.2 Opened control vial for all analytes will be stable for 4 days at 2 — 8 °C, all analytes will be stable for 30 days at -10 to -20 °C
 - 5.3.2.3 Instability or deterioration should be suspected if there are visible signs of leakage, extreme microbial growth or if calibration does not meet the appropriate package insert and/or instrument operation manual criteria.
 - 5.3.3 QC Procedure:
 - 5.3.3.1 Verify that the correct QC values have been entered into the QC file. For details refer to Operator Guide of DimensionEXL200 ,Synchron DXC700 and Atelica CI machines.
 - 5.3.3.2 Allow QC to come to room temperature.
 - 5.3.3.3 Gently remove the stopper to avoid loss of the lyophilized pellet and add exactly 5.0 ml distilled or de-ionized water.

- 5.3.3.4 Leave to stand for 20 minutes.
- 5.3.3.5 Mix bottle several times by inversion to allow homogeneity.
- 5.3.3.6 Gently invert just prior to use. Avoid foaming.
- 5.3.3.7 Open bottle, place a minimum of 1000 ul of each level in separate sample cup, and place on the assigned positions.
- 5.3.3.8 Cap bottle tightly and store at 2-8°C. Immediately after use.
- 5.3.3.9 Perform QC as indicated in Operator Guide of DimensionEXL200 and SynchronDXC600 machines.
- 5.3.4 QC Expected Values:
 - 5.3.4.1 Refer to the Bio-Rad Lyphochek assayed chemistry controls value sheet for DimensionEXL200 ,Synchron DXC700 and Atelica CI machines.

6. RESPONSIBILITIES:

- 6.1 Chemistry shift in charge is responsible for, running calibration and control and samples of direct bilirubin
- 6.2 Chemistry staff are responsible for running direct bilirubin samples all over the day

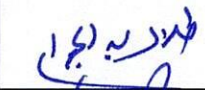

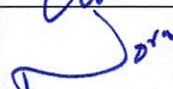
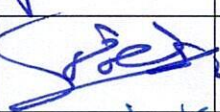

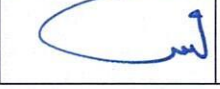
7. APPENDICES:

- 7.1 N/A

8. REFERENCES:

- 8.1 Tietze Text Book of Clinical Chemistry And Molecular Diagnostics 4th Edition,2006
- 8.2 Company Leaflets of reagents

9. APPROVALS:

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