Documented Procedure for Examination of Bilirubin Direct

Purpose of examination:

- Bilirubin Direct estimation from serum or plasma by modified Jendrassik method.

Responsibility and Authority:

- Calibration: Technician
- Quality Control: Technician
- Routine operation: Technician
- Overall Monitoring: Quality Manager

Sample Details:

- Type of Sample: Serum
- Type of container and additives: Plain without any additives
- Patient Preparation: As per Primary Sample Collection Manual
- Stability: Room temperature- 18-28° <24 Hours,
  Refrigerated 2-8° C < 7 days if serum is protected from light
- Handling and transport: As per Primary Sample collection manual
- Storage: 48 hours at 2-8° C

Required Equipment and reagents:

Equipment: Randox Daytona Plus
Accessories and apparatus: NA
Reagents:

R1. Sulphanilic acid	**29.0 mmol/L**
Hydrochloric acid	0.17 mmol/L
R2. Sodium nitrite	**38.5 mmol/L**

Calibration Procedure:

- Name and brand: Randox Calibration serum level 3

Frequency:

- Lot change, due to service or maintenance, system parts are replaced and QC out of range

Procedure:

- **Serum must only be reconstituted using the following procedure:**

1. Open the vial carefully avoiding any loss of material

2. Reconstitute by pipetting exactly 5 ml of distilled water into the vial
3. Replace the stopper and leave to stand for 30 min dark before use
4. Swirl gently several times during the reconstitution period to ensure that the contents are completely dissolved
5. Prior to use, mix the contents by inverting the vial. Do not shake the vial as the information of foam should be avoided. Ensure that no lyophilized material remains un-reconstituted.

Quality control Procedure:

- Name and brand: Randox assayed multisera level 2 and 3
- Procedure / Frequency: 2 Level once a day.
  Procedure as per Quality control procedure (APC/B/Gen/DocPro/7.3.7.1/21)
- Proficiency Testing (EQAS): BIO-RAD

Test Procedure:

Method of the procedure used for examinations

- MODIFIED JENDRASSIK

Principle of the procedure used for examination

Direct bilirubin is determined in the presence of dimethylsulphoxide (DMSO) by the reaction with diazotised sulphanilic acid.

Sample Preparation:

- Serum sample is directly aspirated from the tubes placed in the sample holder
- Sample Volume: 0.1 ml of the sample
- Temperature: 37° C

Procedure steps

- Wait for initialization to complete
- Set power switch at analyzer to on
- Select wash from maintenance screen to wash cuvettes
- Select cell Blank from maintenance screen. (Automated for Randox Daytona Plus)
- Perform photometer check from maintenance screen (Automated for Randox Daytona Plus)
- Prepare reagents to be sufficient for the day

- Level the reagents after registration and re confirm that they are sufficient for the day.
- Prepare and load calibrators and control
- Perform calibration and run control
- Check controls within limit.
- Check samples are free from fibrin.
- Samples are free from bubbles.
- Arrange all the samples in racks/ disc.
- Run labelled samples in the instrument, the results obtained are checked manually.

Performance Characteristics:

- Linearity: 15 mg/dl
- Unit: mg/dl

Normal and critical ranges:

- Reference interval: Adults and infants: 0 - 0.25 mg/dl
- Alert/ critical values: Adults and infants: > 0.2 mg/dl
- Instruction for determining quantitative results when a result is not within the measurement interval
    - If value exceed this linearity limit, dilute the serum with normal saline and repeat the assay, calculate the value using dilution factor.

Laboratory Clinical interpretation:

- Unconjugated hyper-bilirubinemia is seen in newborns, in increased red cell destruction (hemolytic anemia, extension hematoma), is ineffective erythropoiesis some rare genetic disease (Gilbert`s syndrome, Crigler- Najjar syndrome)
- Conjugated hyper-bilirubinemiais associated to a decreases excretion of bile due to liver diasese (hepatitis or cirrhosis) or to intrahepatic or extrahepatic cholestasis
- Hyper-bilirubinemia is seen in jaundice

Interference and cross reaction/potential sources of variation:

- The following analytes were tested up to the levels indicated at Direct Bilirubin concentrations of 0.14mg/dl and 5.03mg/dl, and found not to interfere:

Hemoglobin	No significant interference	up to 1000mg/dl
Triglycerides	No significant interference	up to 750mg/dl
Intralipid®	No significant interference	up to 1000mg/dl
Ascorbic Acid	No significant interference	up to 25mg/dl

Repeat runs Criteria:

- In case of critical values

Reporting time: Routine: 8 hours
For stat; 4 hours

Recording of observation: Software backup
Machine raw data

- **Environment and safety controls:** As per document procedure for post-examination procedure
- **Personal Protection**: As per document procedure for pre-examination procedure
- **Disposal of waste**: As per document procedure for post-examination procedure
- **General environmental caution**: As per document procedure for pre-examination procedure.
- **Literature – reference**: Operators manual kit insert

1. Ehrlich P: Sulfondiazolenzol reagen auf bilirubin. Centr klin Med 4: 721, 1883.
2. Van den Bergh AAH, Muller PP: Uber eine directe und eine indirekte Diazoreaktion auf bilirubin. Biochem Z 77:90, 1916.
3. Winsten S, Cehelyk B: A rapid diazo technique for measuring total bilirubin, Clin Chim Acta 25:441, 1969.
4. Walters MI, Gerarde HW: An ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. Microchem J 15:231, 1970.
5. Gordon ER: The conjugates of bilirubin. Hepatology (NY) 2:19, 1975.
6. Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB Saunders Company; 1995:88-91.

7. Young DS, Effects of Drugs on clinical laboratory tests, AACC Press, Washington, D.C., 1990.