BILIRUBIN DIRECT Assay Kit
JENDRASSIK/GROF METHOD

Cat.No: OttoBC131

Summary & Explanation

80-85% of bilirubin originates on degradation of hemoglobin with the other 15-20% being derived from cytochrome, myoglobin and catalases. Unconjugated bilirubin, which binds to plasma albumin, is produced in the course of degradation in the reticuloendothelial system, liver Kupffer cells, spleen and bone marrow.Unconjugated (primary indirect, water-insoluble) bilirubin is soluble in lipids. With the aid of the glucuronyl transferase enzyme, bilirubin is conjugated primarily by glucuronic acid in the microsomes of hepatic parenchymal cells. In contrast to unconjugated bilirubin, conjugated (secondary, direct) bilirubin is soluble in water, and is excreted via the kidneys. Bilirubin assays are suitable for evaluating the degree of severity of icteric clinical symptoms as well as for monitoring and objectively assessing these symptoms. Distinguishing between direct and indirect bilirubin is a valuable aid in the differential diagnosis of different forms of jaundice. A direct bilirubin value of 50% in hepatic and posthepatic jaundice.

Test Principle

Jendrassik-Gróf method In the presence of caffeine accelerator, total bilirubin couples with sulfanilic acid to form a red azobilirubin dye, the color intensity which is proportional to the bilirubin concentration. Determination of direct bilirubin is performed without caffeine additive. The addition of alkaline tartrate causes a transformation from the red azobilirubin dye to a blue dye and the absorbance maximum from 546nm to 578nm.

Kit Companents

Content	Explanation	Shelf life
Reagent-1 Reagent-2	1x30ml 1x10ml	6 months 6 months
Calibrator	1x0.5ml	6 months
Q.Control	1x0.5ml	6 months

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Calibration and Quality Control: Reconstitute the contents of with 0.5 ml of redistilled water . Stable for 2 days when stored at +4°C

Reactivity

Universal

Specimen

Fresh serum, heparinized plasma or EDTA plasma.
Hemolysis interferes with the test.
Don't use lipemic serum.
Keep out of light and protect the sample from the effects of sunlight.
Centrifuge samples containing precipitate before performing the assay
Stability: 7 days at +2°C - +8°C, 30 days at - 70°C

Assay Range

0.03-13 mg/dl

Refference Range

Each laboratory is recommended to establish their own reference values.

Analytical Performance

Inter Assay Coefficent of Variation (CV) % 2.6
Intra Assay Coefficent of Variation (CV) % 2.5

Procedure

Wavelength	546nm (±10nm)
Sample or Standard	15µl
Reagent-1	300µl
Mix and incubate at room temperature for 5 – 30 minutes. Read absorbance of sample blank.	
Reagent-2	75 µl
Mix and incubate at room temperature for 5 – 30 minutes. Read absorbance of sample against sample blank.	
Zero Adjustment	Sample blank

Calculation

with factor; Concentration mg/dl = 63,1 x ABilirubin Total

with calibrator; Concentration mg/dl = (Asample / Acalibrator) x calibrator conc.

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

References

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