Bilirubin

Reagent for quantitative In-vitro-determination of total bilirubin in serum / plasma

BIL 142

Order No. **BIL 142**
Content: 40 tests

Method
DPD- Method[1)]

Sample material
Serum, heparinized or EDTA plasma.
No use of blood.
Protect sample from action of light.
Diminution at intense action of light: up to 30% / hour.
Haemolysis (Hb > 0.5 g/dL) would interfere.
Stability of bilirubin in serum under exclusion of light:
at +2°C to +6°C: 16 hours
at +15°C to +25°C: 8 hours

Reagents
Content / concentrations:
1. Starter reagent (caps in PE-bottle)
   2.5-Dichlorobenzene diazonium tetrafluoroborat
2. Detergent (pre-portioned in round cuvettes)
   Triton X-100 5%; HCl 100 mmol/L

Safety information
The detergent contains 5 % Triton X-100 and 0.36% hydrochloric acid and is categorized as a dangerous preparation according to EC Directives:
H318: Causes serious eye damage
Observe the safety advice on the packaging.
A safety data sheet is available on request.[2)]
H410: Very toxic to aquatic life with long lasting effects
H290: May be corrosive to metals

Storage and shelf life
The test reagents have to be kept at a temperature between +2°C and +8°C until the expiry date indicated on the packaging. Please take the screw caps out of the container just before the analysis and close the container immediately.

Measurement conditions
Measurement devices: Diaglobal Photometer

Meas. wavelength: 546nm

Temperature: Room temperature

Measurement ranges
BIL: 0,5 - 25 mg/dL (8,50 - 428 µmol/L)
BIL N: 2,30 - 50 mg/dL (39,0 - 850 µmol/L)
In case of exceeding these values, dilute the sample 1+4 with physiological saline solution. Multiply the result by 5. No dilution limit has to be considered with newborn.

Tips
□ When taking this cuvette testing, the sample's blank value and analysis are set into a cuvette and measured one after another.
□ The procedure for newborn (10 µL sample) is recommended only if one expects bilirubin counts of more than 5.0 mg/dL.

Working instructions
A. Determination / adults

Pipette in single test cuvettes BIL 142:	
	Analysis
Sample	100 µL
Mix thoroughly.	

- Select the <BIL> test.
- Insert analysis cuvette (blank value).
- After the signal tone, remove cuvette
- Screw the orange cap onto the cuvette, dissolve the starting reagent powder contained in the cap by inverting several times.
- Press [ON/ENTER].
- Insert analysis cuvette again and wait for result.

B. Determination / newborn

Pipette in single test cuvettes BIL 142:	
	Analysis
Sample	**20 µL**
Mix thoroughly.	

- Select the <BIL N> test.
- Insert analysis cuvette and measure the photometer's zero point A(0).
- Screw caps from the PE bottle onto the cuvettes and dissolve the caps' content by inverting several times, mix thoroughly. Hereby the sample has to be streamed out of the capillary completely.
- Press [ON/ENTER].
- Insert analysis cuvette again and wait for result.

Quality assurance
For quality assurance we recommend universal control sera from company Roche, www.roche.de:
PreciControl ClinChem Multi 1 / Multi 2 (4 x 5 mL)
Order-No.: 05 947 626 190 / 05 947 774 190
Ref.: Roche / Hitachi analyzers, Method: Gen.3 serum, plasma

Reference values[3)]
Total Bilirubin in serum / plasma

		mg/dL	µmol/L
Adults[2)]		up to 1.1	18.8
Newborn[4)]	24 hours	up to 7.0	120
	48 hours	up to 10.3	176
	3rd day	up to 12.7	217
	4th day	up to 13.3	227

Summary[3,4)]
Bilirubin is a degradation product of the haemoglobin. It is carried in the plasma as albumin complex (indirect, unconjugated bilirubin) or as covalent bilirubin which is bound to albumin or rather esterified with glucuronic acid (direct, conjugated). The existent test determines the different bilirubin fractions as total parameters (bilirubin as a whole).

Indications / diagnostic significance:
- diagnosis, differential diagnosis, and progression evaluation of the icterus

The determination of bilirubin as a whole is counted among the basis programme of the examination of newborn.

A direct measurement (direct photometry) of the bilirubin concentration is possible only with newborn because of the plasma's yellow stain. Already a few days after birth, the concentration of the serum carotenes rises and consequently falsifies bilirubin counts which are erroneously too high. The azo methods, which are based on a coupling of bilirubin and a diazotized aromatic amine, have prevailed in the adult diagnosis. They are also qualified for the measurement of the bilirubin of newborn. Besides the quite complex method of Jendrassik-Grof[5)], the newer DPD method has most notably become important.[1)] This method forms the basis of the Diaglobal test.

Measurement principle
In the presence of a detergent with 2.5- dichlorobenzene diazonium salt, bilirubin becomes converted into a red azo dye.

$$\text{Bilirubin} + \text{Diazonium ion} \xrightarrow{\text{Acid}} \text{Azo dye}$$

The intensity of the emerged dye is proportional to the bilirubin concentration in serum / plasma and is measured photometrically.

Attention ! Important Note:

This is the new packing insert for all Diaglobal photometers valid from version V 5.12

The neonatal sample volume has been changed from 10 µL to 20 µL. This results in a change of the factor stored in the photometer as of V 5.12.

We recommend a software update for your device if it does not have the new version V 5.12 yet.

Please contact us.

Performance parameters
Specificity / interferences[3,6)]
Haemoglobin (>0.25 g/L) falsifies too low values. No influence through lipaemia up to 1400 mg/dL. No interference due to ascorbic acid in physiological concentrations (erroneously too low values from 300 mg/L).

Inaccuracy
The reproducibility was checked using human and control samples.

In series [n = 20]	Average [mg/dL]	Standard deviation [mg/dL]	VK [%]
Sample 1	1.02	0.04	4.1
Sample 2	4.80	0.09	1.8
From day to day [n = 20]	**Average [mg/dL]**	**Standard deviation [mg/dL]**	**VK [%]**
Sample 1	1.03	0.04	4.3
Sample 2	4.82	0.09	2.0

Analytic sensitiveness
Lower detection limit: 0.5 mg/dL (8.6 µmol/L)

Comparison of methods
A comparison of the Diaglobal test BIL 142 (y) and a commercially available test (x) resulted in the following correlation according to the Passing/Bablok[7)] process:
y = 0.952x + 0.026
r = 0.993
n = 45
Concentration range: 0.5 - 40 mg/dL

Information on disposal
Waste code number 180106:
Vials with reagent are considered hazardous waste. Do not allow reagent to reach surface water or sewage system.
Dispose of in accordance with official regulations.
Non-contaminated and completely empty packaging can be recycled.