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Bilirubin Total, Jendrassik Grof

(en) English

REF	Content
103107B	1 x 1 L R1 + 1 x 0.04 L R2
102001	5 x 100 mL R1 + 1 x 20 mL R2
102011	5 x 50 mL R1 + 1 x 10 mL R2
116021	5 x 25 mL R1 + 1 x 5 mL R2

For professional in vitro diagnostic use only.

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of total bilirubin in human serum or plasma on photometric systems.

DIAGNOSTIC SIGNIFICANCE

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucuronic acid and the resulting water-soluble bilirubin glucuronic acid is excreted via the bile ducts. Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60 – 70% of neonates due to an increased postpartum breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated water soluble bilirubin. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin[1,2].

TEST PRINCIPLE

Bilirubin is converted in the liver to bilirubin monoglucuronide and bilirubin diglucuronide. The reaction of bilirubin with diazotized sulfanilic acid was first described by Ehrlich[3] and was subsequently used by Van den Bergh[4,5] to provide the first method for the quantification of bilirubin in normal and pathological serum samples. Modifications and improvements of this reaction, in acid and alkaline medium have been described by many authors.
Free bilirubin is not soluble in aqueous solution and requires solubilization by alcohols or other solvents to react. Reactions carried out in these solvents provide measurements of total bilirubin. Mono and diglucuronides of bilirubin are water soluble and measurements performed in aqueous solution measure what in this form is called direct bilirubin.
In our reagents we use dimethylsulfoxide (DMSO) and ethylene glycol as solvents for the total bilirubin assay. Bilirubin in these solvents readily reacts with diazotized sulfanilic acid to produce an intensely colored diazo dye. The intensity of color of this dye in solution is proportional to the concentration of total bilirubin.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1	
Sulfanilic Acid	32.2 mmol/L
Ethylene Glycol	
Dimethylsulfoxide (DMSO)	
Reagent 2	
Sodium Nitrite	109 mmol/L

MATERIAL REQUIRED BUT NOT PROVIDED

- Calibrator, e.g.:

REF	Name	Content
D98485	Diacal Auto	5 x 3 mL
D98485SV	Diacal Auto	1 x 3 mL

- Controls, e.g.:

REF	Name	Content	Description
D98481	Diacon N	12 x 5 mL	Control normal
D14481	Diacon N	5 x 5 mL	Control normal
D98481SV	Diacon N	1 x 5 mL	Control normal
D98482	Diacon P	12 x 5 mL	Control abnormal
D14482	Diacon P	5 x 5 mL	Control abnormal
D98482SV	Diacon P	1 x 5 mL	Control abnormal

- Clinical chemistry analyser.
- General laboratory equipment.

REAGENT PREPARATION

Substrate Start:
Reagents are ready to use.

Sample Start:
Mix 150 parts of Reagent 1 with 1 part of Reagent 2.
(= working reagent)

STORAGE AND STABILITY

Conditions: Protect from direct light.
Avoid contamination.
Close immediately after use.
Do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8 °C
Stability: up to the expiration date

Sample Start (Working Reagent):
Stability: at 20 – 25 °C 8 hours*

*In amber bottles.

WARNINGS AND PRECAUTIONS

1. Components contained in Bilirubin Total, Jendrassik Grof are classified according to EC regulation 1272/2008 (CLP) as follows:

   Reagent 1: Danger

   Contains sulfanilic acid. May produce an allergic reaction.
   H314: Causes severe skin burns and eye damage.
   P260: Do not breathe dusts or mists.
   P280: Wear protective gloves/eye protection/face protection.
   P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
   P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
2. The reagent R2 contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Please refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patients' medical history, clinical examinations and other findings.
5. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
6. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
7. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Human serum or plasma.

It is very important to store the sample protected from light!
Use only clear non-hemolyzed serum.

Stability[6]:

In serum/plasma	at 20 – 25 °C	2 days
	at 4 – 8 °C	7 days
	at - 20 °C	6 months*

*In case of immediate freezing.
Freeze only once! Discard contaminated specimens.

TEST PROCEDURE

Method: Endpoint, Jendrassik Grof, DMSO, colorimetric
Wavelength: 555 nm
Optical path: 1 cm
Temperature: 20 – 25 °C, 37 °C
Measurement: Against respective blank. Increasing reaction.

Bring reagents and samples to room temperature.

Sample Start:

Pipette into test tubes	Sample blank	Sample	Calibrator blank	Calibrator
Reagent 1	1000 µL	-	1000 µL	-
Working Reagent	-	1000 µL	-	1000 µL
Sample	100 µL	100 µL	-	-
Calibrator	-	-	100 µL	100 µL
Mix without delay. Incubate for 3 minutes at 30 °C or for 2 minutes at 37 °C. Read absorbance of each test against the respective blank.				

Substrate Start:

Pipette into test tubes	Sample blank	Sample	Calibrator blank	Calibrator
Reagent 1	1000 µL	1000 µL	1000 µL	1000 µL
Sample	100 µL	100 µL	-	-
Calibrator	-	-	100 µL	100 µL
Reagent 2	-	10 µL	-	10 µL
Mix without delay. Incubate for 3 minutes at 30 °C or for 2 minutes at 37 °C. Read absorbance of each test against the respective blank.				

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation

With calibrator:

$$\text{Bilirubin [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Calibrator [mg/dL]}$$

With factor (light path 1 cm)**:**
From absorbance readings calculate ΔA and multiply by the corresponding factor:

Bilirubin [mg/dL] = ΔA Sample x Factor

Factor: 12 .9

The factor has to be checked by a calibration serum and adapted if necessary!

Unit Conversion
Bilirubin [mg/dL] x 17.1 = Bilirubin [µmol/L]

QUALITY CONTROL AND CALIBRATION

We recommend the DIALAB serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range). All target values of the controls are traceable to DIALAB reagent/calibrator system. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery. Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
We recommend the DIALAB multi calibration serum **Diacal Auto**.

PERFORMANCE CHARACTERISTICS

Precision (at 37°C)

Intra-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.80	0.01	1.82
Sample 2	1.16	0.02	1.32
Sample 3	4.69	0.02	0.45

Inter-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.82	0.02	2.38
Sample 2	1.13	0.02	1.56
Sample 3	4.61	0.03	0.71

Analytical sensitivity

The lower limit of detection is 1 mg/dL.

Linearity and measuring range

The assay is linear to 20 mg/dL.
Samples with bilirubin concentrations higher than 20 mg/dL should be diluted with distilled or deionized water and the assay should be repeated; multiply results by dilution factor.

Analytical specificity

no interference up to:

Hemoglobin 1000 mg/dL
For further information on interfering substances refer to Young DS[7].

Clinical performance

A comparison between DIALAB Bilirubin Total, Jendrassik Grof (y) and a commercially available test (x) using 55 samples gave following results: y = 0.989 x + 0.001 mg/dL; r= 0.998.

TRACEABILITY

The assigned values of Diacal Auto have been made traceable to molar extinction coefficient 546 nm.

EXPECTED VALUES

	[mg/dL]	[µmol/L]
Neonates[1]		
24h	< 8.8	< 150
2nd day	1.3 – 11.3	22 – 193
3rd day	0.7 – 12.7	12 – 217
4th – 6th day	0.1 – 12.6	1.7 - 216
Children[1]	0.2 – 1.0	3.4 – 17
>1 month		
Adults[1]	0.1 – 1.2	1.7 - 21

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATIONS

- Although verification and validation studies have examined the effect of likely interferents in this assay, there is a potential for interference from unknown substances. Therefore, as with any diagnostic test, is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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USED SYMBOLS

Symbol	Description
Cont.	Content