Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12151	R1	1 x	40	ml
For 100 tests Total	R2	1 x	10	ml
For 100 tests Direct	R3	2 x	50	ml
	R4	2 x	50	ml
Cat. No. 12152	R1	1 x	80	ml
For 200 tests Total	R2	1 x	20	ml
For 200 tests Direct	R3	2 x	100	ml
	R4	2 x	100	ml

Bilirubin Total & Direct

Colorimetric, Jendrassik-Grôf method

Liquid Reagents

Test principle

Sulfanilic acid reacts with sodium nitrite to form diazotized Sulfanilic acid. In this step we determined direct bilirubin at 550 nm. In the presence of caffeine "accelerator", total bilirubin couples with diazotized Sulfanilic acid to give an azo dye that measured at 580nm, the color intensity of which is proportional to the bilirubin concentration.

Concentrations in the test

Reagent R1		
Sulfanilic acid	28.9	mmol/L
hydrochloric acid	170	mmol/L
Reagent R2		
Sodium nitrite	25	mmol/L
Reagent R3		
Caffeine	260	mmol/L
Sodium benzoate	520	mmol/L
Reagent R4		
Tartarate	0.93	mol/L

Stability of reagents

Reagent R1: *liquid, ready to use.*
Reagent R2: *liquid, ready to use.*
Reagent R3: *liquid, ready to use.*
Reagent R4: *liquid, ready to use.*

All reagents are stable up to expiry date given on label when stored at +20 ➔ +25 °C.

Always close bottles firmly after use.
Avoid direct light exposure of all reagents.
Reagent required but not provided: 0.9 % sodium chloride solution.

Note: Don't use if sodium nitrite reagent develops to a dark yellow discoloration.

Specimen collection and handling

1. Fresh non-hemolyzed serum is recommended.
2. Plasma collected EDTA, heparin, citrate or fluoride.
3. Sample stability 2 hours at 20 - 25 °C, 12 hours at 2 - 8 °C, and 3 months at - 20 °C.
4. Keep samples away from light and sunlight.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	T. Bili. Hg 578 nm (560 - 600 nm) D. Bili. Hg 546 nm (530 - 560 nm)
Spectrophotometer	T. Bili. 580 nm D. Bili. 550 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against assay blank
Reaction	end point

Assay

1. **Total Bilirubin**

	Sample Blank	Sample	Paediatric Blank	Paediatric
Reagent R1	100 µl	100 µl	100 µl	100 µl
Reagent R2	--	25 µl	--	25 µl
Reagent R3	500 µl	500 µl	500 µl	500 µl
Sample	100 µl	100 µl	20 µl	20 µl
Mix, incubate for 5 min. at 37 °C or 10 min. at 20 – 25 °C.				
Reagent R4	500 µl	500 µl	500 µl	500 µl
Mix, incubate for 5 min. at 37 °C or 10 min. at 20 - 25 °C. Read the absorbance against assay blank (A_{assay}). The color is stable for 1 hour.				

2. **Direct Bilirubin**

	Sample Blank	Sample
Reagent R1	100 µl	100 µl
Reagent R2	--	25 µl
NaCl 0.9 %	1000 µl	1000 µl
Sample	100 µl	100 µl
Mix, Read the absorbance against assay blank (A_{assay}) exactly after 5 min. at 20 - 25 °C, or 3 min. at 37 °C. The color is stable for 15 minutes.		

Calculation

Calculation using Factor:

$Conc._{Bilirubin}$ (mg/dl) = A_{Sample} X Factor

	T. Bilirubin	D. Bilirubin	T. Bilirubin **Paediatric**
Wavelength	578 nm	546 nm	578 nm
Factor	**10.8**	**14.4**	**50**

Calculation using Calibrator:

$$Conc._{T\text{-}Bilirubin}\ (mg/dl) = \frac{A_{Sample}}{A_{Calibrator}} \times Conc._{Calibrator}\ (mg/dl)$$

$$Conc._{D\text{-}Bilirubin}\ (mg/dl) = \frac{A_{Sample}}{A_{Calibrator}} \times Conc._{Calibrator}\ (mg/dl)$$

Note: Each laboratory could make its own factor, under standarized conditions, for each lot of the reagent by using the following formula:

$$F = \frac{Conc._{Calibrator}}{A_{Calibrator}}$$

$$\mu mol/L \xrightarrow{X\ 0.0585} mg/dl \qquad mg/dl \xrightarrow{17.1\ X} \mu mol/L$$

Linearity

T.Bil. up to 20 mg/dl (340 µmol/L).
D.Bil. up to 10 mg/dl (170 µmol/L).
If the result exceeds 20 mg/dl, repeat the test by using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. A number of drugs and substances affect Bilirubin results. See Young, *et al*.
2. Hemolysis interferes with the test. Usually low values are obtained.
3. Lipemia causes false high values.
4. Light and sunlight cause false low value. Direct sunlight may cause up to 50 % decrease in Bilirubin within one hour.
5. Hepatotoxic drugs which cause cholestasis and Hemolysis produce elevated recoveries.

Precautions

Reagents are toxic and corrosive.
Don't pipette by mouth.
Avoid contact with skin and clothing.

Reference range

Total Bilirubin

1 d.	< 5.0	mg/dl
2 d.	< 9.0	mg/dl
3 - 5 d.	< 12	mg/dl
Children	< 1.5	mg/dl
Adults	< 1.1	mg/dl

Direct Bilirubin

Adults	< 0.3	mg/dl

References

1. Jendrassik , Biochem,Z. 297 : 81 (1938),
2. Seymour Winsten, Clin. Chem. Acta. 24, 441 - 446 (1969).
3. Martinek, R. G., Clin. Chem. Acta 13 : 161 (1966).
4. Kees L. J. Vink, Clin. Chem. 34/1, 67 - 70 (1988).
5. Kees L. J. Vink, Clin. Chem. 32/7, 1986 - 1393 (1986).
6. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
7. Tietz , N. W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Sauders Company, p. 54 (1983).
8. Clinical laboratory diagnostics: Laboratory results /ed. By Lothar Thomas - 1. ed. -Frankfurt/main: TH-books-verl. -Ges., 1998 Einheitssacht.: Labor und Diagnose < engl. > ISBN 3 - 9805215 - 4 - 0 P:201.
9. Jendrassik L, Gróf P. Simplified photometric methods for the determination of bilirubin. Biochem Zschr 1938, 297: 8 1 - 9.
10. National Committee for Clinical Laboratory Standards. Reports on analyte reference summaries of the National Reference System for Clinical Laboratory. NRSCL, 7-CR. Villanova: NCCLS, 1989.

Medichem Middle East Aleppo, Syria, E-mail: info@medichem-me.com