

Manual Procedure

Cat. No. 12161 For 100 tests	R1	1 ×	50 ml
	R2	1 ×	50 ml
	R3	2 ×	50 ml

Bilirubin Total (DCA)

Colorimetric test, DCA method

liquid reagents

Test Principle

Dichloroaniline (DCA) reacts with nitrite to give a diazo compound, which reacts with bilirubin to form azobilirubin. The detergent liberates the indirect bilirubin bound to albumin.

Concentrations in the test

Reagent R1			
Dichloroaniline	30.9	mmol/L	
HCl	80,0	mmol/L	
Detergent			
Reagent R2			
Sodium nitrite	25	mmol/L	
Reagent blank R3			
Dichloroaniline	15.4	mmol/L	
HCl	40,0	mmol/L	

Stability and preparation of working reagent

Reagent R1: liquid,
Reagent R2: liquid,
Reagent R3: liquid, ready for use.

Working Reagent (R1+R2)

Mix reagent R1 and reagent R2 at the ratio 1 to 1,. Working reagent is ready for use after 30 min. Stability: 4 weeks at 2 – 8 °C
All reagents are stable up to expiry date given on label when stored at 2 – 8 °C. Avoid direct light exposure.

Note: Do not use if sodium nitrite reagent develops a dark yellow discoloration.

Specimen Collection and Handling

- Fresh non-hemolyzed serum is recommended.
- Plasma collected in EDTA, heparin, citrate or fluoride.
- Sample stability 2 hour at 20 – 25 °C
12 hours at 2 – 8 °C
3 months at – 20°C
- Keep sample 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	Hg 546 nm
Spectrophotometer	550 nm
Cuvette	1 cm light path
Temperature	20 – 25 °C
Measurement	against assay blank
Reaction	End point – Sample blank

Assay

	Sample / calibrator		Pediatric	
	Blank	Assay	Blank	Assay
Sample / calibrator	100 µl	100 µl	20 µl	20 µl
Reagent R3	1000 µl	--	1000 µl	--
Working Reagent	--	1000 µl	--	1000 µl

Mix, incubate for 10 min. at 20 – 25°C. Read absorbance against assay blank (A_{assay}). Color is stable for 60 minutes in the dark.

Calculation

$$Conc. \text{ T-Bilirubin} = \frac{A_{\text{Sample}}}{A_{\text{Calibrator}}} \times Conc. \text{ Calibrator}$$

Or multiply the absorbance by a factor (F):

	Assay	Pediatric assay
Total bilirubin mg/dl	A X 12.5	A X 58
Total bilirubin µmol/L	A X 214	A X 992

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

$$F = \frac{Conc. \text{ Calibrator}}{\Delta A \text{ Calibrator}}$$

$$\mu\text{mol/L} \xrightarrow[1.71 \times]{\times 0.585} \text{mg/L}$$

Linearity

Up to 30 mg/dl (510 µmol/L)
If result exceeds 30 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9%) and multiply result by 2.

Interference

- A number of drugs and substances affect bilirubin results. see Young, *et al.*⁶
- Hemolysis interferes with the test. Usually low values are obtained.
- Lipemia causes falsely high values.
- Light and sunlight cause falsely low value. Direct sunlight may cause up to a 50% decrease in bilirubin within one hour.
- Hepatotoxic drugs which cause cholestasis and hemolysis produce elevated level.

Precautions

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

Total Bilirubin DCA colorimetric method

Reference range

Adult/Children	Up to 1,1 mg/dl
Newborn	1 day old: up to 7,0 mg/dl
	2 days old: up to 10,3 mg/dl
	3 days old: up to 12,7 mg/dl
	4 days old: up to 13,3 mg/dl

References

1. Seymour Winsten, Clin. Chem. Acta. 24, 441-446 (1969).
2. Martinek, R. G., Clin. Chem. Acta 13:161 (1966).
3. Kees L. J. Vink, Clin. Chem. 34/1, 67-70 (1988).
4. Kees L. J. Vink, Clin. Chem. 32/7, 1986-1393 (1986).
5. Tietz , N. W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders Company, p. 54 (1983).
6. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
7. Jendrassik L, Gróf P. Simplified photometric methods for the determination of bilirubin. Biochem Zschr 1938; 297: 8 1-9.
8. National Committee for Clinical Laboratory Standards. Reports on analyte reference summaries of the National Reference System for Clinical Laboratory. NRSL, 7-CR. Villanova: NCCLS, 1989.