

BILIRUBIN

JENDRASSIK & GROF METHOD

Principle:

Bilirubin in the presence of a Sulphanilic Acid diazonium salt forms a red coloured azo compound in alkaline solutions. The total and direct bilirubin in serum is determined using the method of Jendrassik and Grof by coupling it with diazotised sulphanilic Acid after the addition of caffeine, sodium benzoate.

Reagent Concentration:

Reagent 1. Sulphanilic acid solution:
Sulphanilic acid 30mmol/L

Reagent 2. Sodium nitrite solution:
Sodium nitrite 50mmol/L

Reagent 3. Caffeine solution:
Caffeine 100mmol/L

Preparation and Stability:

All reagents are ready to use.
Stability up to the expiry date stated when sealed and stored refrigerated(+2 to +8 °C).
Always keep bottles tightly closed.

Samples:

For use with serum or plasma samples
Haemolysis interferes with the test.
Do not expose sample to sunlight or other light.
Samples can be stored for up to 3 months at -20 C, 4 days at 2-8 C and 1 day at 15-25 C

Procedure:

Wavelength: Hg 546 (540nm)
Temperature: +20 to +25°C
Cuvette: 1cm light path
Zero adjustment: Against sample blank

	Total	Direct	Blank
R1	100µl	100µl	100µl
R2	25µl	25µl	--
Saline	--	1.0ml	1.0ml
R3	1.0ml	--	--
Sample	100µl	100µl	100µl

Mix. Let stand for 5 minutes at +20-+25°C.
Read absorbance of total and direct samples.

Calculation:

Absorbance tube Total x 17.5 = Total Bilirubin (mg/dl)

Absorbance tube Direct x 17.5 = Direct Bilirubin (mg/dl)

(mg/dl) x 17.1 = µmol/L

Linearity:

The method is linear up to 20mg/dl (342µmol/l).
In case of higher results, dilute sample 1:2 with NaCl 0.9% solution and repeat test. Multiply result by 2.

Normal Values:

Serum:
Total Bilirubin up to 1.1mg/dl (18.8µmol/l)
Direct Bilirubin up to 0.25mg/dl (4.3µmol/l)

Notes:

This kit does not include a standard.

Quality Control:

For accuracy and reproducibility control:-
Assayed Multi-Sera Normal and Elevated.
For reproducibility control:-
Multi-Sera Loaw, Normal and Elevated.

Presentation:

BIL0152, 150 tests
Caffeine (RIII) 1x150ml
Sulphanilic Acid (RI) 1x60ml
Sodium Nitrite (RII) 1x10ml

Safety precautions:

For in vitro diagnostic use only. Do not pipette by mouth.
Exercise the normal precautions required for handling laboratory reagents.

Health and Safety data sheets are available on request.

Literature:

Jendrassik, L. et al. Biochem. Z. 297:81 (1938)
Malloy, H.T. and Evelyn, K.A., J. Biol. Chem. 119, 481 (1937)
Matimek, R.G. Clin Chem. Acta, 13 161 (1966)