BILIRUBIN TOTAL / DIRECT COMBO (15-25°C)

JENDRASSIK GROF

CATALOGUE NUMBER	KIT SIZE (ml)
MPRBIL1	2x30ml / 2x6ml / 2x60ml / 2x60ml
MPRBIL2	1x120ml / 1x24ml / 1x250ml / 1x250ml

Intended Use:
For *In Vitro* diagnostic use by trained professionals only.
This reagent is intended for the quantitative determination of Total and Direct Bilirubin in serum and plasma.

Clinical Significance:
80-85% of Bilirubin originates from degradation of haemoglobin with the other 15-20% being derived from cytochrome, myoglobin and catalases. Unconjugated bilirubin which is transported bound to plasma albumin is produced by degradation in the reticuloendothelial system, liver Kupffer cells, spleen and bone marrow. Bilirubin assays are useful in evaluating the degree of severity of icteric clincal symptoms as well as for monitoring these symptoms. A direct bilirubin value of <20% of the total bilirubin value is an indicator of jaundice of pre-hepatic origin. The ratio can increase to more than 50% in hepatic and post-hepatic jaundice.

Test Principle:
Jendrassik Grof method. In the presence of caffeine acclerator, total bilirubin couples with diazotised sulphanilic acid to form a red azobilirubin dye, the intensity of which is proportional to the total bilirubin concentration. Direct bilirubin is determined in the same reaction but without the caffeine accelerator.

Reagent Composition:

R1: Sulphanilic Acid	Sulphanilic Acid 29 mmol/l Hydrochloric Acid 0.17 mol/l
R2: Sodium Nitrite	Sodium Nitrite: 25 mmol/l
R3: Caffeine	Caffeine 0.26 mol/l Sodium Benzoate 0.52 mol/l
R4: Tartrate	Tartrate 0.93 mol/l Sodium Hydroxide 1.9 mol/l

Precautions and Warnings:
Exercise normal precautions required for handling all laboratory reagents.
R4 contains sodium hydroxide which is highly caustic. Wear protective gloves and safety glasses when working with the reagents.

Reagent Preparation and Stability:
All reagents are provided ready to use.
Stability: Up to expiry date at 15 - 25°C.
Dispose of reagents carefully in line with local guidelines.

Sample Collection, Preparation and Stability:
Collect serum and heparin or EDTA plasma by standard venepuncture technique. Haemolysis interferes in the assay.
Protect samples from light at all stages from collection. Perform assay immediately.

Calibration:
If the assays cannot be performed using 578nm or 546nm wavelengths, run using a suitable calibrator.

Assay Procedure: TOTAL BILIRUBIN: Room temperature: 15 - 25°C.

WAVELENGTH	578 nm
TEMPERATURE	37°C
CUVETTE	1cm Path Length
BLANK	DDH_2O

	Sample Blank	Sample
R1 – Sulphanilic Acid	200 µl	200 µl
R2 – Sodium Nitrite	-	50 µl
R3: Caffeine	1000 µl	1000 µl
Sample	200 µl	200 µl
Mix and incubate at room temperature for 10 minutes, then add		
R4: Tartrate	1000 µl	1000 µl
Mix and incubate for 5 minutes at room temperature. Measure absorbance of sample against the sample blank.		

Calculation:
Abs Total Bilirubin = Abs sample – Abs Sample Blank
Total Bilirubin (mg/dl) (578nm) = 10.8 x Abs Total Bilirubin
Total Bilirubin (µmol/l) (578nm) = 185 x Abs Total Bilirubin

Assay Procedure: DIRECT BILIRUBIN

WAVELENGTH	546 nm
TEMPERATURE	37°C
CUVETTE	1cm Path Length
BLANK	DDH_2O

	Sample Blank	Sample
R1 – Sulphanilic Acid	200 µl	200 µl
R2 – Sodium Nitrite	-	50 µl
Normal Saline	2000 µl	2000 µl
Sample	200 µl	200 µl
Mix and incubate for 5 minutes at room temperature. Measure absorbance of sample against the Sample Blank.		

Calculation:
Abs Direct Bilirubin = Abs sample – Abs Sample Blank
Direct Bilirubin (mg/dl) (546nm) = 14.4 x Abs Direct Bilirubin
Direct Bilirubin (µmol/l) (546nm) = 246 x Abs Direct Bilirubin

Performance Characteristics:

Measuring range:
0 - 25 mg/dl
Dilute samples with higher concentrations using Normal saline 1+4 and rerun the assay. Multiply the result by the dilution factor (for 1+4 dilution, the dilution factor is 5)

Analytical Sensitivity: (Lowest detection limit):
This is limited by the sensitivity of the photometer used. In most manual photometers the sensitivity is 0.18 mg/dl of total bilirubin and 0.24 mg/dl of Direct bilirubin

Imprecision
Intra-Assay Precision:

Sample	Mean (mg/dl)	SD (mg/dl)	CV %
Level 1	1.26	0.04	3.10%
Level 2	5.78	0.09	1.55%

Inter-Assay Precision:

Sample	Mean (mg/dl)	SD (mg/dl)	CV %
Level 1	1.32	0.03	2.27%
Level 2	5.75	0.10	1.74%

Interferences:
Haemolysis interferes with the test giving low values. Protect samples from light to avoid falsely low values. Lipaemia causes falsely high values.

Reference Range:

(Adult)	
Total Bilirubin	</= 1.0 mg/dl (17 µmol/l)
Direct Bilirubin	</= 0.25 mg/dl (4.3 µmol/l)

Each laboratory should establish its own mean reference range according to the population.

Limitations:
The result from this test should not be used as the sole criteria for diagnosis, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

Automated systems:
Contact AMS Technical Department for applications on a wide range of automated analysers.
For automation we recommend the use of a serum based calibrator.

Quality Control and Calibration Material:
Calibration Serum: **QCCCAL1 / QCCCAL2**
Human Assayed Control Normal: **QCCHAN1 / QCCHAN2**
Human Assayed Control Elevated: **QCCHAE1 / QCCHAE2**

References:

1. Thomas L ed Labor und Diagnose, 4th ed Marburg: Die Medizinische Verlafsgesellschaft 1992.
2. Jendrassik L et al. Biochem Z 1938; 297: 81

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

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