DIRECT BILIRUBIN (D BIL)

Colorimetric Method
RX SERIES

INTENDED USE

For the quantitative *in vitro* determination of Direct Bilirubin in serum and plasma. This product is suitable for use on RX **series** instruments which includes the RX **daytona** and the RX **imola**.

Cat. No.			
BR 3807	R1.	Saline	2 x 30 ml
	R2a.	Sulphanilic acid	8 x 4.0 ml
	R2b.	Nitrite Reagent	1 x 2 ml

GTIN: 05055273200836

CLINICAL SIGNIFICANCE[1]

A bilirubin (total or direct) test system is a device intended to measure the levels of bilirubin (total or direct) in plasma or serum. Measurements of the levels of bilirubin, an organic compound formed during the normal and abnormal disruption of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

PRINCIPLE[2]

Direct bilirubin is coupled with diazotized sulfanilic acid to form an azo dye.

This assay uses an endpoint method and 2 point calibration.

SAMPLE COLLECTION AND PREPARATION[1]

Serum, heparinized plasma or EDTA-plasma. Samples are stable for 3 days at room temperature protected from light.

REAGENT COMPOSITION

Contents		Initial Concentration of Solutions
R1.	**Saline:**	
	NaCl	9 g/l
R2a.	**Sulphanilic acid**	29 mmol/l
	Hydrochloric acid	170 mmol/l
R2b.	**Sodium nitrite**	385 mmol/l

SAFETY PRECAUTIONS AND WARNINGS

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

Nitrite Reagent contains sodium nitrite at a concentration which is harmful.

Health and Safety Data Sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1 Saline
Contents supplied ready for use. Stable up to the expiry date specified when stored at +15 to +25°C.

R2 Diazo reagent
Shortly before use add 1 drop of Nitrite to 1 vial of Sulphanilic acid. Mix well.

MATERIALS PROVIDED

Saline
Sulphanilic acid
Sodium nitrite

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multi-sera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532).
Randox Calibration Serum Level 3 (Cat. No. CAL 2351).

PROCEDURE NOTES

To avoid the potential for reagent carryover, it is recommended that the testing order of the reagents is confirmed. Please consult the reagent carryover document available on www.randox.com under support and documentation - Reagent product inserts or by contacting Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

The Chemistry Parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyzer PC. The required programs should be downloaded to the analyzer software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue contact Randox Laboratories Technical services, Northern Ireland +44 (0) 28 94451070.

CALIBRATION

The use of Randox Calibration Sera Level 3 is recommended for calibration. A blank is recommended every day.

QUALITY CONTROL

Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services Northern Ireland +44 (0) 28 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

INTERFERENCE

Haemolysis interferes with the test. Fresh samples should be used and kept out of direct light.

The analytes below were tested up to the following levels and were found not to interfere:

Haemoglobin	<250 mg/dl
Triglycerides	1000 mg/dl
Intralipid®	<200 mg/dl

Studies have been performed to determine the level of interference from the following substances. The criteria for no significant interference are recovery within ±10% of the initial value of Direct Bilirubin concentration of 2.4 µmol/l and 86 µmol/l.

	2.4 µmol/l	**86 µmol/l**
Phenlybutazone	3.1 mg/l	31.5 mg/l
3-Indoxylsulfate Potassium Salt	13 mg/dl	13 mg/dl
Indo green	1.11 mg/l	2.1 mg/l

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [3]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

NORMAL VALUES IN SERUM[4]

Direct bilirubin:	up to 4.3 µmol/l
	up to 2.5 mg/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data was obtained using an RX **daytona** analyser at 37°C.

LINEARITY

The method is linear up to 137 µmol/l (81.1 mg/l). In the event of a rerun, the linearity is extended to 275 µmol/l (162 mg/l).

SENSITIVITY

The minimum detectable concentration of direct bilirubin with an acceptable level of precision was determined as 1.42 µmol/l.

PRECISION

Within run precision

	Level 1	Level 2	Level 3
Mean (µmol/l)	9.90	18.6	32.1
SD	0.155	0.235	0.503
CV(%)	1.57	1.26	1.57
n	20	20	20

Between run precision

	Level 1	Level 2	Level 3
Mean (µmol/l)	4.29	19.1	34.9
SD	0.181	0.462	0.746
CV(%)	4.22	2.24	2.14
n	20	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.03 X - 0.40
and a correlation coefficient of r = 1.00

50 patient samples were analyzed spanning the range 1.47 to 144 µmol/l.

REFERENCES

1. Lothar, T. ed., Clinical Laboratory Diagnostics, 1st Edition, TH-Books Verlagsgesellschaft mbH, Frankfurt/Main, Germany (1998) p. 198.
2. Jendrassik, L. and Grof. P., Biochem. Z. 1938; **297**: 81.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.
4. Sherlock, S.,(1951), Liver Disease, Churchill London, page 204.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

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