TOTAL BILIRUBIN 2 (T BIL 2)

VANADATE OXIDATION METHOD
RX DAYTONA PLUS

INTENDED USE

For the quantitative *in vitro* determination of Total Bilirubin in serum and plasma. This product is suitable for use on the RX **daytona plus**.

FOR PRESCRIPTION USE ONLY

Cat. No.			
BR 8377	R1.	Total Bilirubin 2 R1	4 x 20 mL
	R2.	Total Bilirubin 2 R2	4 x 8 mL

GTIN: 05055273214772

CLINICAL SIGNIFICANCE

Total Bilirubin measurements are used in the diagnosis and treatment of haemolytic, biliary and liver disorders including hepatitis and cirrhosis. Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucuronic acid to form a soluble compound. This conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. An unconjugated, albumin bound form is also present in the circulation. It is insoluble and does not normally pass through the kidneys into the urine.
An increase in bilirubin concentration in the serum or tissues is called jaundice. Jaundice occurs in toxic or infectious diseases of the liver e.g. hepatitis B or obstruction of the bile duct and in rhesus incompatible babies.

Useful information may be obtained by determining which form of bilirubin is elevated.

High levels of conjugated or direct bilirubin indicate that bile is not being properly excreted; therefore, an obstruction may be present in the bile duct or gall bladder. Unconjugated or indirect bilirubin can also be determined by subtracting the direct bilirubin level from the total bilirubin result. High levels of unconjugated bilirubin indicate that too much haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving.

PRINCIPLE [1]

The bilirubin is oxidised by vanadate at about pH 2.9 to produce biliverdin. In the presence of detergent and vanadate, both conjugate (direct) and unconjugated bilirubin are oxidised. This oxidation reaction causes a decrease in the optical density of the yellow colour, which is specific to bilirubin. The decrease in optical density at 450/546 nm is proportional to the total bilirubin concentration in the sample. The concentration is measured as an endpoint reaction.

Bilirubin + Surfactant + VO^{3-} ⟶ Biliverdin

SAMPLE COLLECTION AND PREPARATION [2]

Serum or plasma (lithium heparin). Samples should be protected from light.
Serum specimens are stable for 3 months when stored frozen at -70°C with no light exposure.

REAGENT COMPOSITION

Contents			Initial Concentration of Solutions
R1.	**Total Bilirubin 2 R1**		
	Citrate buffer, pH2.9		0.1 mol/L
	Detergent		0.9%
R2.	**Total Bilirubin 2 R2**		
	Phosphate buffer, pH 7.0		10 mmol/L
	Sodium Metavanadate		4 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.

This kit contains components classified as hazardous in accordance with the Regulation (EC) No.1272/2008:
R2

WARNING
Causes serious eye irritation.

Precautions:
Wear protective gloves/ protective clothing/ eye protection/face protection.
Wash thoroughly after handling.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If eye irritation persists: Get medical advice/attention.

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

Reagents are supplied ready to use. Stable up to expiry when stored at +2°C to +8°C. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use. The reagent is stable for 28 days on-board the RX daytona plus analyser.

MATERIALS PROVIDED

Total Bilirubin 2 R1
Total Bilirubin 2 R2

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)
RX **series** Saline (Cat. No. SA 8396)

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 **daytona plus** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser 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 9445 1070.

RX DAYTONA PLUS CALIBRATION

0.9% NaCl as zero calibrator and Randox Calibration Serum Level 3 are recommended for calibration. A 2 point calibration is recommended every 28 days with a change of reagent lot or as indicated by the quality control procedures.

STANDARDISATION

Randox Calibration Serum Level 3 is traceable to an internal master reference material that is traceable to NIST SRM916(a).

QUALITY CONTROL

Randox Assayed Multisera, 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 9445 1070.

NORMAL VALUES IN SERUM [3]

Total Bilirubin	5 – 21	µmol/L
	0.3 – 1.2	mg/dL

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data was obtained using an RX **daytona plus** analyser.

SERUM INTERFERENCE

Studies have been performed to determine the level of interference from the following substances. The criteria for no significant interference is recovery within ±10% of the initial value of Total Bilirubin concentration of 16.1 µmol/L (0.940 mg/dL) and 258 µmol/L (15.1 mg/dL).

Haemoglobin	No significant interference up to	330 mg/dL
Ascorbic Acid	No significant interference up to	50 mg/dL
Intralipid	No significant interference up to	750 mg/dL
Triglycerides	No significant interference up to	2000 mg/dL

PLASMA INTERFERENCE

The criteria for no significant interference is recovery within ±10% of the initial value of Total Bilirubin concentration of 16.1 µmol/L (0.940 mg/dL) and 258 µmol/L (15.1 mg/dL).

	16.1 µmol/L	**258 µmol/L**
Intralipid	143 mg/dL	750 mg/dL

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 [4]. 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.

REPORTABLE RANGE

Linearity data demonstrates that the reportable range for Total Bilirubin on the RX **daytona plus** is 2.61 to 578.98 µmol/L (0.153 to 33.9 mg/dL).

LINEARITY

Linearity was determined in accordance with C.L.S.I. standard EP6-A. The method is linear to 578.98 µmol/L (33.9 mg/dL)

SENSITIVITY

The limit of Quantitation (LoQ), the limit of Detection (LoD) and the limit of Blank (LoB) were determined consistent with CLSI guidelines EP17-A2. LoQ is the lowest concentration that can be detected with ≤20% imprecision. LoD is the lowest concentration that can be detected to determine the presence or absence of Total Bilirubin. LoB is the highest concentration that is likely to be observed in a blank sample.

RX daytona plus

	µmol/L	mg/dl
Limit of Blank	0.04	0.002
Limit of Detection	1.00	0.059
Limit of Quantitation	2.61	0.153

PRECISION

Precision estimates were completed according to CLSI documents EP5-A2. Each sample was assayed in duplicate twice per day for 20 days.

Within Run Precision

		Level 1	Level 2	Level 3	Level 4
Mean	(µmol/L)	15.25	23.61	39.69	255.45
	(mg/dL)	0.892	1.38	2.32	14.9
SD	(µmol/L)	0.33	0.26	0.48	2.53
	(mg/dL)	0.019	0.015	0.028	0.148
CV	(%)	2.2	1.1	1.2	1.0
n		80	80	80	80

Total Precision

		Level 1	Level 2	Level 3	Level 4
Mean	(µmol/L)	15.25	23.61	39.69	255.45
	(mg/dL)	0.892	1.38	2.32	14.9
SD	(µmol/L)	0.39	0.32	0.61	2.89
	(mg/dL)	0.023	0.019	0.036	0.169
CV	(%)	2.6	1.4	1.5	1.1
n		80	80	80	80

CORRELATION

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

µmol/L: $Y = 1.06X - 0.57$
mg/dL: $Y = 1.06X - 0.033$
and a correlation coefficient of $r = 0.998$

180 patient samples were analysed spanning the range 3.74 to 558.02 µmol/L (0.219 – 32.6 mg/dL).

MATRIX COMPARISON

Lithium Heparin

Patient samples were drawn in matched pairs – one sample serum (x) and the second sample lithium heparin plasma (y). A minimum of 75 matched patient sample pairs were analysed in singlicate spanning the range 2.72 to 579.71 µmol/L and the following linear regression equation was obtained:

$y = 1.00 \times 1.00$
Correlation coefficient of $r = 0.998$

REFERENCES

1. Tokuda K, Tanimoto K. New method of measuring serum bilirubin using vanadic acid. *Jpn J Clin Chem.* 1993:22:116-122.
2. Tietz Fundamentals of Clinical Chemistry. 5th ed. Burtis CA, Ashwood ER, eds. Philadelphia, PA: WB Saunders Company; 2001:605.
3. WuAHB. Tietz Clinical Guide to laboratory Tests, 4th edition, Saunders Elsevier, St. Louis, MO: 2006:316.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

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

Revised 23 Apr 24 ld

THIS PAGE IS INTENTIONALLY BLANK