BILT2

cobas®

Bilirubin Total DPD Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03146022 122	Bilirubin Total DPD Gen.2 250 tests	System-ID 07 6587 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
10158046 122	Precibil (4 x 2 mL)	Code 306	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

BILT2: ACN 257

SBIL2: ACN 275 (STAT, reaction time: 5)

For **cobas c** 502 analyzer:

BILT2: ACN 8257

SBIL2: ACN 8275 (STAT, reaction time: 5)

Intended use

In vitro test for the quantitative determination of total bilirubin in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1]

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract.

Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Test principle

Colorimetric assay.[2]

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a diazonium ion in a strongly acidic medium.

$$\text{Bilirubin + diazonium ion} \xrightarrow{\text{acid}} \text{azobilirubin}$$

The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

Reagents - working solutions

R1 Detergent; hydrochloric acid: 120 mmol/L

R2 3,5-dichlorophenyl diazonium salt: ≥ 1.5 mmol/L

R1 is in position B, and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

BILT2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K_2-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems

from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability:[a,3] 1 day at 15-25 °C
7 days at 2-8 °C
6 months at (-15)-(-25) °C

a) If care is taken to prevent exposure to light

Materials provided
See "Reagents – working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section
General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 6-17 (STAT 5 / 6-17)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	124 µL	–	
R2	25 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	2 µL	–	–

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-25 (STAT 5 / 10-25)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	124 µL	–	
R2	25 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	2 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-25 (STAT 5 / 10-25)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	124 µL	–	
R2	25 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: The method has been standardized against the Doumas method.[4]

Quality control
For quality control, use control materials as listed in the "Order information" section.
In addition, other suitable control material can be used.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation
Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: µmol/L x 0.0585 = mg/dL
mg/dL x 10 = mg/L
mg/dL x 17.1 = µmol/L

Limitations - interference
Criterion: Recovery within ± 10 % of initial value at the indicated total bilirubin concentration.
Hemolysis:[5] No significant interference up to an H index of 50 (approximate hemoglobin concentration: 31 µmol/L or 50 mg/dL) at a total bilirubin concentration of 17 µmol/L (1.0 mg/dL). No significant interference in neonates up to an H index of 400 (approximate hemoglobin concentration: 248 µmol/L or 400 mg/dL) at a total bilirubin concentration of 150 µmol/L (8.8 mg/dL).
Lipemia (Intralipid):[5] No significant interference up to an L index of 300 at a total bilirubin concentration of 17 µmol/L or 1.0 mg/dL. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[6,7]
Cyanokit (Hydroxocobalamin) may cause interference with results.
Indican: No significant interference from indican up to levels of 3 mg/dL or 30 mg/L at a total bilirubin concentration of 17 µmol/L (1.0 mg/dL).
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[8]
Results from certain multiple myeloma patients may show a positive bias in recovery. Not all multiple myeloma patients show the bias and the severity of the bias may vary between patients.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both D-bilirubin and total bilirubin values.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

1.7-650 µmol/L (0.1-38.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Recommended dilution of samples via the rerun functions is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit

1.7 µmol/L (0.1 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Adults[9]	up to 21 µmol/L	(up to 1.2 mg/dL)
Children with age ≥ 1 month[9]	up to 17 µmol/L	(up to 1.0 mg/dL)

Reference range study with 500 well-characterized human serum samples: [10]

Males	up to 24 µmol/L	(up to 1.4 mg/dL)
Females	up to 15 µmol/L	(up to 0.9 mg/dL)

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[11]

Age of newborn:

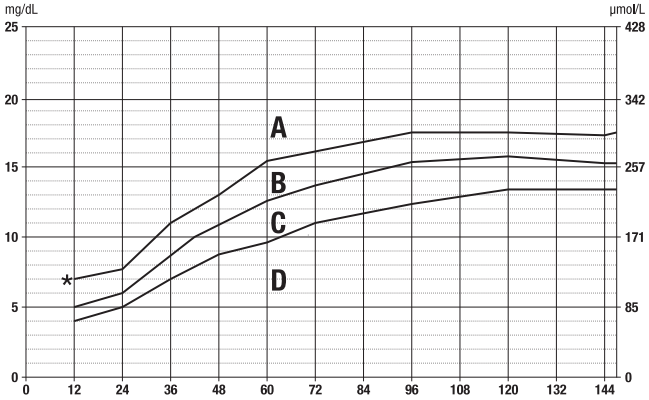
24 hours	≥ 137* µmol/L	(≥ 8.0* mg/dL)
48 hours	≥ 222* µmol/L	(≥ 13.0* mg/dL)
84 hours	≥ 290* µmol/L	(≥ 17.0* mg/dL)

*95th percentile

Levels > 95th percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

Roche has not evaluated reference ranges in a pediatric population.

Nomogramm for designation of risk in 2840 well newborns[11]
Serum Bilirubin



Postnatal Age (hours)

* 95th percentile

A High risk zone
B High intermediate risk zone
C Low intermediate risk zone
D Low risk zone

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean* µmol/L (mg/dL)	*SD* µmol/L (mg/dL)	*CV* %
Precinorm U	19.1 (1.12)	0.5 (0.03)	2.5
Precipath U	80.7 (4.72)	0.4 (0.02)	0.5
Human serum 1	13.8 (0.807)	0.4 (0.023)	2.7
Human serum 2	117 (6.85)	1(0.06)	0.6
Intermediate precision	*Mean* µmol/L (mg/dL)	*SD* µmol/L (mg/dL)	*CV* %
Precinorm U	19.9 (1.16)	0.4 (0.02)	2.0
Precipath U	70.6 (4.13)	1.8 (0.11)	2.6
Human serum 3	9.53 (0.560)	0.43 (0.025)	4.5
Human serum 4	176 (10.3)	2 (0.1)	1.4

Method comparison

Total bilirubin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 150

Passing/Bablok[12]	Linear regression
y = 0.996x - 1.562 µmol/L	y = 0.994x - 0.750 µmol/L

$r = 0.980$ $r = 0.998$

The sample concentrations were between 3.20 and 456 µmol/L (0.187 and 26.7 mg/dL).

References

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11 Subcommittee on Hyperbilirubinemia. Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation. Pediatrics 2004;114:297-316.

12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

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