

BILT3

Bilirubin Total Gen.3



Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
05795397 190	Bilirubin Total Gen.3 250 tests	System-ID 07 7483 9
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
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3

English

System information

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

BILT3: ACN 712

SBIL3: ACN 711 (STAT, reaction time: 4)

For **cobas c** 502 analyzer:

BILT3: ACN 8712

SBIL3: ACN 8711 (STAT, reaction time: 4)

Intended use

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

Summary¹

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 principle²

Colorimetric diazomethod

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium.



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 Phosphate: 25 mmol/L; detergents; stabilizers, pH 1.0

R2 3,5-dichlorophenyl diazonium salt: ≥ 1.35 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.

This kit contains components classified as follows in accordance with the European directive 1999/45/EC:



C-Corrosive

- R 35 Causes severe burns.
- S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
- S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

Reagent handling

Ready for use

Storage and stability

BILT3

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, K₂-, K₃-EDTA plasma

(The use of EDTA-plasma with elevated hematocrit may lead to slightly lower values.)

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 4 / 6-17)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	120 µL	–	
R2	24 µL	–	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	8 µL	15 µL	105 µL
Increased	2 µL	–	–

cobas c 501 test definition

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-25 (STAT 4 / 10-25)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	120 µL	–	
R2	24 µL	–	

Sample volumes

	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	8 µL	15 µL	105 µL
Increased	2 µL	–	–

cobas c 502 test definition

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-25 (STAT 4 / 10-25)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	120 µL	–	
R2	24 µL	–	

Sample volumes

	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	8 µL	15 µL	105 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H ₂ O
	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 was 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 × 0.0585 = mg/dL
	mg/dL × 10 = mg/L
	mg/dL × 17.1 = µmol/L

Limitations - interference

Criterion: Recovery within ± 3.4 µmol/L (0.199 mg/dL) of initial values of samples ≤ 34 µmol/L (1.99 mg/dL) and ± 10 % of samples > 34 µmol/L.

Hemolysis:⁵ No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Criterion: Recovery within ± 1.7 µmol/L (0.099 mg/dL) of initial values of samples ≤ 17 µmol/L (0.995 mg/dL) and ± 10 % of samples > 17 µmol/L.

Hemolysis in neonates:⁵ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

BILT3

Bilirubin Total Gen.3

cobas®

Lipemia (Intralipid):⁵ No significant interference up to an L index of 1000. 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}

Indican: No significant interference from indican up to levels of 0.12 mmol/L or 3 mg/dL.

Cyanokit (Hydroxocobalamin) may cause falsely low results.

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.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.⁸

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

2.5-650 µmol/L (0.146-38.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

LoB = 1.7 µmol/L (0.099 mg/dL)

LoD = 2.5 µmol/L (0.146 mg/dL)

LoQ = 2.5 µmol/L (0.146 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration bilirubin samples.

Values below the Limit of Quantitation will not be flagged by the instrument.

In the USA, when reporting values below 2.5 µmol/L (0.146 mg/dL), the client report should be annotated with the following information, "Values below 2.5 µmol/L (0.146 mg/dL) are not reliable due to possible higher uncertainty."

Expected values

Adults⁹ up to 21 µmol/L (up to 1.2 mg/dL)

Children with age ≥ 1 month 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-term¹¹

Age of newborn:

24 hours ≥ 137 µmol/L^a (≥ 8.0 mg/dL^b)

48 hours ≥ 222 µmol/L^b (≥ 13.0 mg/dL^b)

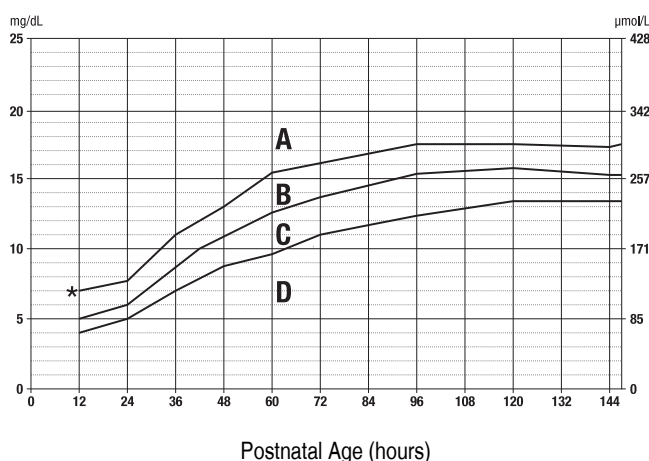
84 hours ≥ 290 µmol/L^b (≥ 17.0 mg/dL^b)

a) 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.

Nomogramm for designation of risk in 2840 well newborns¹¹

Serum Bilirubin



* 95th percentile

A High risk zone

C Low intermediate risk zone

B High intermediate risk zone

D Low risk zone

Roche has not evaluated reference ranges in a pediatric population.

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

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	Mean	SD	CV
	µmol/L (mg/dL)	µmol/L (mg/dL)	%
Control level 1	15.4 (0.901)	0.3 (0.018)	2.1

Control level 2	52.8 (3.09)	0.3 (0.02)	0.6
Human serum A	8.69 (0.508)	0.25 (0.015)	2.9
Human serum B	302 (17.7)	2 (0.1)	0.6
Human serum C	544 (31.8)	2 (0.1)	0.4
<i>Intermediate precision</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	$\mu\text{mol/L (mg/dL)}$	$\mu\text{mol/L (mg/dL)}$	%
Control level 1	15.4 (0.901)	0.3 (0.018)	2.1
Control level 2	52.8 (3.09)	0.4 (0.02)	0.8
Human serum A	8.69 (0.508)	0.29 (0.017)	3.3
Human serum B	302 (17.7)	2 (0.1)	0.8
Human serum C	544 (31.8)	3 (0.2)	0.6

Method comparison

Total bilirubin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) using the Roche Bilirubin Total Gen.3 reagent were compared with those determined on a COBAS INTEGRA 800 analyzer using the same reagent (x).

Sample size (n) = 64

Passing/Bablok¹² Linear regression

$$y = 0.995x + 0.734 \mu\text{mol/L} \quad y = 0.993x + 1.20 \mu\text{mol/L}$$

$$\tau = 0.990 \quad r = 1.00$$

The sample concentrations were between 3.6 and 618 $\mu\text{mol/L}$ (0.211 and 36.2 mg/dL).

Total bilirubin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) using the Roche Bilirubin Total Gen.3 reagent were compared with those determined using the Roche Total Bilirubin Special reagent on the same analyzer (x).

Sample size (n) = 152

Passing/Bablok¹² Linear regression

$$y = 0.962x + 1.55 \mu\text{mol/L} \quad y = 0.936x + 3.01 \mu\text{mol/L}$$

$$\tau = 0.981 \quad r = 1.00$$

The sample concentrations were between 2.4 and 561 $\mu\text{mol/L}$ (0.140 and 32.8 mg/dL).

References

- 1 Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987;729-761.
- 2 Wahlefeld AW, Herz G, Bernt E. Modification of the Malloy-Evelyn method for a simple, reliable determination of total bilirubin in serum. Scand J Clin Lab Invest 1972;29 Supplement 126:Abstract 11.12.
- 3 Quality of Diagnostic Samples, Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and Laboratory medicine, 3rd completely revised ed. 2010.
- 4 Dumas BT, Kwok-Cheung PP, Perry BW, et al. Candidate Reference Method for Determination of Total Bilirubin in Serum: Development and Validation. Clin Chem 1985;31:1779-1789.
- 5 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 6 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- 7 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
- 8 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

- 9 Thomas L, ed. Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die Medizinische Diagnostik, 7th ed.: TH-Books Verlagsgesellschaft 2007:259-273.
- 10 Löhr B, El-Samali V, Junge W, et al. Reference Range Study for Various Parameters on Roche Clinical Chemistry Analyzers. Clin Lab 2009;55:465-471.
- 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

GTIN

Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS C, COBAS INTEGRA, PRECIBIL, PRECINORM, PRECIPATH and PRECICONTROL are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

Significant additions or changes are indicated by a change bar in the margin.

© 2014, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

