BILT3

Bilirubin Total Gen.3

cobas®

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056960190	08056960500	Bilirubin Total Gen.3 (1050 tests)	System-ID 2031 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
10158046122	Precibil (4 x 2 mL)	Code 20306	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

BILT3: ACN 20310

Intended use

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

Summary

Measurements of total bilirubin, performed with this assay in human serum and plasma of adults and neonates, are used for the diagnosis of hyperbilirubinemia (such as observed with abnormal destruction of red blood cells, liver diseases, and metabolic disorders, including hepatitis and gallbladder block), and in newborn screening for severe hyperbilirubinemia. Total bilirubin is a combination of direct and indirect bilirubin.

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.[1,2,3,4]

Numerous guidelines, including those from the World Health Organization, the American College of Gastroenterology, and National Institute for Health and Care Excellence, recommend bilirubin testing as part of the diagnostic workup for liver injury.[3,4,5,6,7]

In newborns, several mechanisms lead to an increased bilirubin load, such as increased turnover in fetal red blood cells, reduced bilirubin clearance, and increased enterohepatic circulation of bilirubin. Screening neonates for severe hyperbilirubinemia, especially in newborns with infant jaundice, has been proposed to help preventing chronic bilirubin encephalopathy.[8,9] For infants born at >= 35 weeks of gestation, the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia recommends to measure total serum bilirubin in case of jaundice in the first 24 hours after birth or if jaundice appears excessive for infants' age (all bilirubin levels should be interpreted according to the infant's age in hours).[8]

Test principle[10]

Colorimetric diazo method

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

$$\text{Bilirubin} + \text{3,5-DPD} \xrightarrow{\text{acid}} \text{azobilirubin}$$

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 Phosphate: 50 mmol/L; detergents; stabilizers; pH 1.0

R3 3,5-dichlorophenyl diazonium salt: ≥ 1.35 mmol/L

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

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

Danger

H290	May be corrosive to metals.
H319	Causes serious eye irritation.
H360FD	May damage fertility. May damage the unborn child.

Prevention:

P201	Obtain special instructions before use.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P308 + P313	IF exposed or concerned: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P390	Absorb spillage to prevent material damage.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.
Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

BILT3

Bilirubin Total Gen.3

cobas®

Storage and stability

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

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-, K_3-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.

See the limitations and interferences section for details about possible sample interferences.

Stability:[a),11]	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

Freeze only once.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	600/546 nm		
Reagent pipetting		Diluent (H_2O)	
R1	78 µL	–	
R3	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.3 µL	–	–
Decreased	2.6 µL	20 µL	60 µL
Increased	1.3 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

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

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: The method was standardized against the Doumas method.[12]

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 6 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample in the unit µmol/L (mg/dL, mg/L).

Conversion factors:	µmol/L x 0.0585 = mg/dL
	µmol/L x 0.585 = mg/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 within ± 10 % for samples > 34 µmol/L.

Hemolysis:[13] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Immunoglobulins: No significant interference from immunoglobulins up to a concentration of 28 g/L (187 µmol/L) (simulated by human immunoglobulin G).

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

Hemolysis in neonates:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[13] 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.[14,15]

Indican: No significant interference from indican up to a concentration of 0.12 mmol/L (3 mg/dL).

Cyanokit (Hydroxocobalamin) may cause falsely low results.

Samples containing indocyanine green must not be measured.

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.[16]

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions refer to the operator's manual.

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, Limit of Detection and Limit of Quantitation

Limit of Blank	= 1.7 µmol/L (0.099 mg/dL)
Limit of Detection	= 2.5 µmol/L (0.146 mg/dL)
Limit of Quantitation	= 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.

Expected values

µmol/L

Adults[17]	up to 21 µmol/L
Children with age ≥ 1 month[17]	up to 17 µmol/L

Reference range study with 500 well-characterized human serum samples:[18]

Males	up to 24 µmol/L
Females	up to 15 µmol/L

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[19]

Age of newborn:

24 hours	≥ 137 µmol/L[b)]
48 hours	≥ 222 µmol/L[b)]
84 hours	≥ 290 µmol/L[b)]

b) 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.

mg/dL

Adults[17]	up to 1.2 mg/dL
Children with age ≥ 1 month[17]	up to 1.0 mg/dL

Reference range study with 500 well-characterized human serum samples:[18]

Males	up to 1.4 mg/dL
Females	up to 0.9 mg/dL

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[19]

Age of newborn:

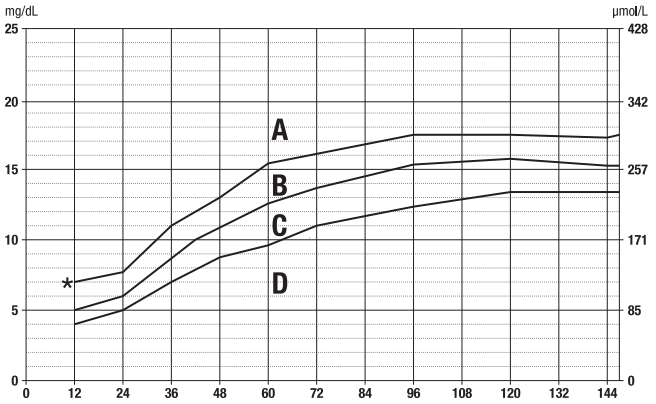
24 hours	≥ 8.0 mg/dL[b)]
48 hours	≥ 13.0 mg/dL[b)]
84 hours	≥ 17.0 mg/dL[b)]

b) 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.

Nomogram for designation of risk in 2840 well newborns[19]

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
PCCC1[c)]	16.2	0.256	1.6
PCCC2[d)]	61.4	0.315	0.5
Human serum 1	5.43	0.211	3.9
Human serum 2	21.5	0.228	1.1

Human serum 3	91.6	0.507	0.6
Human serum 4	295	1.24	0.4
Human serum 5	519	1.97	0.4
Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
PCCC1[c)]	16.2	0.372	2.3
PCCC2[d)]	60.9	0.630	1.0
Human serum 1	5.43	0.222	4.1
Human serum 2	21.4	0.269	1.3
Human serum 3	91.6	0.706	0.8
Human serum 4	295	1.57	0.5
Human serum 5	516	3.26	0.6

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

Total bilirubin values for human serum and plasma samples obtained with the Roche Bilirubin Total Gen.3 reagent on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 649

Passing/Bablok[20]	Linear regression
y = 1.000x - 0.0394 µmol/L	y = 1.002x - 0.339 µmol/L
τ = 0.979	r = 1.000

The sample concentrations were between 2.51 and 622 µmol/L.

Total bilirubin values for human serum and plasma samples obtained with the Roche Bilirubin Total Gen.3 reagent on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 67

Passing/Bablok[20]	Linear regression
y = 1.010x - 0.247 µmol/L	y = 1.008x - 0.264 µmol/L
τ = 0.966	r = 1.000

The sample concentrations were between 2.90 and 615 µmol/L.

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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cobas®

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche