0105795419190c701V10.0

BILT3

Bilirubin Total Gen.3

cobas®

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05795419 190	Bilirubin Total Gen.3 (600 tests)	System-ID 05 7483 9	**cobas c** 701/702

Materials required (but not provided):

10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 122	Precipath U plus (10 x 3 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	
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English

System information

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.

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[2]

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.

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

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 (STAT R2)	3,5-dichlorophenyl diazonium salt: ≥ 1.35 mmol/L

R1 is in position B and R3 (STAT R2) 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.
H314	Causes severe skin burns and eye damage.
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:

P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
P308 + P313	IF exposed or concerned: Get medical advice/attention.

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

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
On-board on the Reagent Manager:	24 hours

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:	4 weeks
On-board on the Reagent Manager:	24 hours

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,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 701/702 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 18-27 (STAT 4 / 6-15)		
Wavelength (sub/main)	600/546 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R3 (STAT R2)	24 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	8 µL	13 µL (STAT 15 µL)	110 µL (STAT 105 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

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

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

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

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 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.[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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

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

Limits and ranges

Measuring range

BILT3, ACN 8712:

2.5-550 µmol/L (0.146-32.2 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.37 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.37.

SBIL3, ACN 8711 (STAT):

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.

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

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:

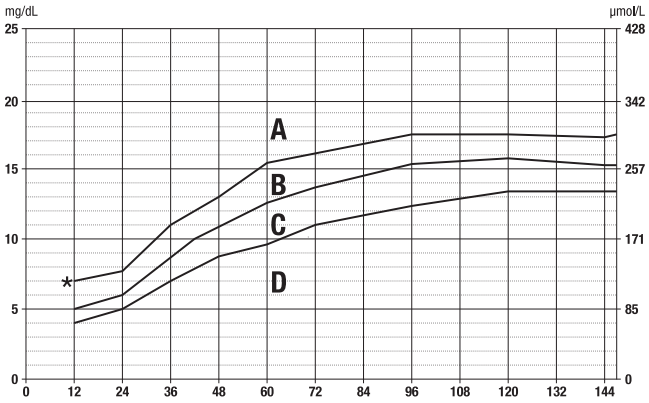
24 hours	≥ 137 µmol/L[b]	(≥ 8.0 mg/dL[b])
48 hours	≥ 222 µmol/L[b]	(≥ 13.0 mg/dL[b])
84 hours	≥ 290 µmol/L[b]	(≥ 17.0 mg/dL[b])

[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.

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

Repeatability was determined using human samples and controls in an internal protocol (n = 21, 1 run). Intermediate precision was 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	15.7 (0.918)	0.2 (0.012)	1.2
Control level 2	53.1 (3.11)	0.3 (0.02)	0.6
Human serum A	9.06 (0.530)	0.23 (0.013)	2.5
Human serum B	310 (18.1)	1 (0.1)	0.4
Human serum C	460 (26.9)	3 (0.2)	0.7
Intermediate precision	*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)	0.4 (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

Results for intermediate precision were obtained on the master system **cobas c** 501 analyzer.

BILT3

Bilirubin Total Gen.3

cobas®

Method comparison

Total bilirubin values for human serum and plasma samples obtained on a **cobas c** 701 analyzer using the Roche Bilirubin Total Gen.3 reagent (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 61

Passing/Bablok[12]	Linear regression
y = 0.994x - 0.069 µmol/L	y = 0.993x - 0.011 µmol/L
τ = 0.988	r = 1.00

The sample concentrations were between 4.1 and 519 µmol/L (0.240 and 30.4 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 Doumas 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-Samalouti 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.

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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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