BILT3

Bilirubin Total Gen.3

cobas®

Substrates

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	COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
10171778 122	Precipath U (20 × 5 mL)	System-ID 07 7998 9	
10171760 122	Precipath U (4 × 5 mL)	System-ID 07 7998 9	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
10158046 122	Precibil (4 × 2 mL)	System-ID 07 6604 6	

English

System information

Test BILT3, test ID 0-712

Intended use

In vitro test for the quantitative determination of total bilirubin in serum and plasma of adults and neonates on COBAS INTEGRA 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.

Bilirubin + 3,5-DPD —*acid*→ azobilirubin

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

Reagents - working solutions

R1 Phosphate: 25 mmol/L; detergents; stabilizers; pH 1.0

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

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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.

Prevention:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or doctor/physician.

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.

P390 Absorb spillage to prevent material damage.

Product safety labeling primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	

On-board in use at 10-15 °C	6 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	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, 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.

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.

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.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	33/46
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	120 µL	0 µL
Sample	2 µL	0 µL
SR	24 µL	0 µL
Total volume	146 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	44/65
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	120 µL	0 µL
Sample	2 µL	0 µL
SR	24 µL	0 µL
Total volume	146 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Recommended
Calibration interval	Each lot and as required following quality control procedures.

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

Quality control

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus, Precibil or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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 INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factors:	µmol/L × 0.0585 = mg/dL
	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 in adults:[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).

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] Exception: Cyanokit (Hydroxocobalamin) may cause falsely low results.

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

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 direct 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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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, 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[10] with 500 well-characterized human serum samples

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:

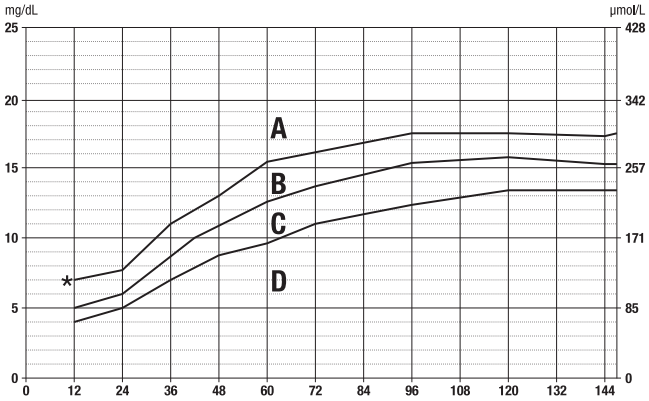
24 hours	≥ 137 µmol/L[b)]	(≥ 8.0 mg/dL[b)])
48 hours	≥ 222 µmol/L[b)]	(≥ 13.0 mg/dL[b)])
84 hours	≥ 290 µmol/L[b)]	(≥ 17.0 mg/dL[b)])

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.

Nomogram for designation of risk in 2840 well newborns[11]

Serum Bilirubin



***** 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 COBAS INTEGRA 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 µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control level 1	16.0 (0.936)	0.3 (0.018)	2.1
Control level 2	53.7 (3.14)	0.5 (0.03)	0.9
Human serum A	8.90 (0.521)	0.44 (0.026)	4.9
Human serum B	308 (18.0)	2 (0.1)	0.6
Human serum C	555 (32.5)	3 (0.2)	0.6

Intermediate precision	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control level 1	16.0 (0.936)	0.4 (0.023)	2.5
Control level 2	53.7 (3.14)	0.8 (0.05)	1.4
Human serum A	8.90 (0.521)	0.46 (0.027)	5.2
Human serum B	308 (18.0)	6 (0.4)	2.1
Human serum C	555 (32.5)	11 (0.6)	1.9

Method comparison

Total bilirubin values for human serum and plasma samples obtained on a COBAS INTEGRA 800 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) = 64

Passing/Bablok[12]	Linear regression

y = 1.005x - 0.738 µmol/L | y = 1.006x - 1.13 µmol/L

τ = 0.990 | r = 1.00

The sample concentrations were between 4.1 and 610 µmol/L (0.240 and 35.7 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.

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

CE

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Roche