

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

English**System information**

Test BILT2, test ID 0-587

Intended use

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

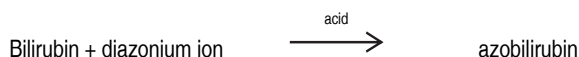
Summary¹

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 assay

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



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** Hydrochlorid: 120 mmol/L**SR** 3,5-dichlorophenyl diazonium salt: ≥ 1.5 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.

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, K₂-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.

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 ₂ O)
R1	110 µL	0 µL
Sample	2 µL	8 µL
SR	24 µL	0 µL
Total volume	144 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 ₂ O)
R1	110 µL	0 µL
Sample	2 µL	8 µL
SR	24 µL	0 µL
Total volume	144 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 factor: µmol/L × 0.0585 = mg/dL

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a total bilirubin concentration of 17 µmol/L (1.0 mg/dL).

Serum/plasma

Hemolysis from adults:⁵ No significant interference up to an H index of 140 (approximate hemoglobin concentration: 87 µmol/L or 140 mg/dL).

Lipemia (Intralipid):⁵ No significant interference up to an L index of 1300. 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} Exceptions: Hydroxocobalamin (Cyanokit) may cause false-low results.

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

Hemolysis from neonates: No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL at a total bilirubin concentration of 150 µmol/L or 8.8 mg/dL).

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**

1.7-650 µmol/L (0.1-38.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function 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 of the test:

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 3 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-term¹¹

Age of newborn:

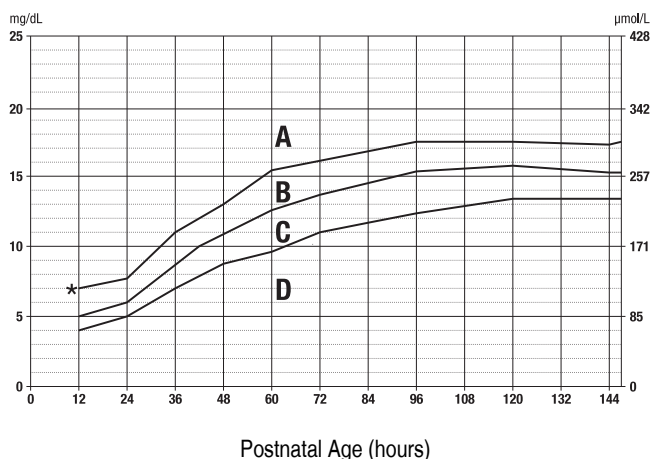
24 hours	≥ 137* µmol/L	(≥ 8.0* mg/dL)
48 hours	≥ 222* µmol/L	(≥ 13.0* mg/dL)

84 hours $\geq 290^* \mu\text{mol/L}$ ($\geq 17.0^* \text{mg/dL}$)*95th percentile

Levels $\geq 95^{\text{th}}$ 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

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

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 $\mu\text{mol/L}$ (mg/dL)	SD $\mu\text{mol/L}$ (mg/dL)	CV %
Precinorm U	19.3 (1.13)	0.5 (0.03)	2.4
Precipath U	88.8 (5.20)	0.6 (0.04)	0.7
Human serum 1	15.5 (0.91)	0.5 (0.03)	3.1
Human serum 2	164 (9.61)	1 (0.06)	0.6

Intermediate precision	Mean $\mu\text{mol/L}$ (mg/dL)	SD $\mu\text{mol/L}$ (mg/dL)	CV %
Precinorm U	19.5 (1.14)	0.7 (0.04)	3.4
Precipath U	87.5 (5.12)	1.4 (0.08)	1.6
Human serum 1	15.0 (0.88)	0.7 (0.04)	4.7
Human serum 2	161 (9.44)	3 (0.18)	1.7

Method comparison

Total bilirubin values for human serum and plasma samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the corresponding Bilirubin DPD (BIL-T) reagent on a Roche/Hitachi 917 analyzer (x). Sample size (n) = 74

Passing/Bablok¹²

Linear regression

 $y = 0.989x - 0.340 \mu\text{mol/L}$ $r = 0.982$

SD (md 95) = 2.74

The sample concentrations were between 2.60 and 462 $\mu\text{mol/L}$ (0.152 and 27.0 mg/dL).

Total bilirubin values for human serum and plasma samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the corresponding Bilirubin DPD (BIL-T) reagent on a COBAS INTEGRA 800 analyzer (x). Sample size (n) = 73

Passing/Bablok¹² $y = 1.000x - 1.67 \mu\text{mol/L}$ $r = 0.967$

SD (md 95) = 2.79

The sample concentrations were between 3.35 and 406 $\mu\text{mol/L}$ (0.196 and 23.8 mg/dL).

 $y = 0.988x - 0.307 \mu\text{mol/L}$ $r = 1.00$

Sy.x = 1.65

Linear regression

 $y = 1.000x - 1.98 \mu\text{mol/L}$ $r = 1.00$

Sy.x = 2.04

References

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- 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

BILT2

Bilirubin Total DPD Gen.2

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

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