

**Bilirubin Total Gen.3****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
05795397 190	Bilirubin Total Gen.3 (250 tests)	System-ID 07 7483 9 Roche/Hitachi <b>cobas c</b> 311, <b>cobas c</b> 501/502
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
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.

**Summary<sup>1</sup>**

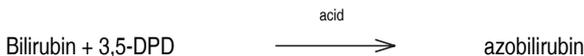
Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated 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<sup>2</sup>**

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:  $\geq 1.35$  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 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 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. + P331

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

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

P305 + P351 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 or doctor/physician. + P310

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**

**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, K<sub>2</sub><sup>-</sup>, K<sub>3</sub>-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:<sup>a,3</sup> 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 <sub>2</sub> O)		
R1	120 µL	–	
R2	24 µL	–	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
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 <sub>2</sub> O)		
R1	120 µL	–	
R2	24 µL	–	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
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 <sub>2</sub> O)		
R1	120 µL	–	
R2	24 µL	–	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	2 µL	–	–
Decreased	8 µL	15 µL	105 µL
Increased	4 µL	–	–

### Calibration

Calibrators	S1: H <sub>2</sub> O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration <ul style="list-style-type: none"> <li>after reagent lot change</li> <li>as required following quality control procedures</li> </ul>

Traceability: The method was standardized against the Doumas method.<sup>4</sup>

### 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

$$\text{mg/dL} \times 17.1 = \mu\text{mol/L}$$

**Limitations - interference**

Criterion: Recovery within  $\pm 3.4 \mu\text{mol/L}$  (0.199 mg/dL) of initial values of samples  $\leq 34 \mu\text{mol/L}$  (1.99 mg/dL) and  $\pm 10\%$  of samples  $> 34 \mu\text{mol/L}$ .

Hemolysis:<sup>5</sup> No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497  $\mu\text{mol/L}$  or 800 mg/dL).

Criterion: Recovery within  $\pm 1.7 \mu\text{mol/L}$  (0.099 mg/dL) of initial values of samples  $\leq 17 \mu\text{mol/L}$  (0.995 mg/dL) and  $\pm 10\%$  of samples  $> 17 \mu\text{mol/L}$ .

Hemolysis in neonates:<sup>5</sup> No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621  $\mu\text{mol/L}$  or 1000 mg/dL).

Lipemia (Intralipid):<sup>5</sup> 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.<sup>6,7</sup>

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.

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.<sup>8</sup>

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-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  $\mu\text{mol/L}$  (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  $\mu\text{mol/L}$  (0.099 mg/dL)

Limit of Detection = 2.5  $\mu\text{mol/L}$  (0.146 mg/dL)

Limit of Quantitation = 2.5  $\mu\text{mol/L}$  (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 95<sup>th</sup> percentile value from  $n \geq 60$  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**

Adults<sup>9</sup> up to 21  $\mu\text{mol/L}$  (up to 1.2 mg/dL)

Children with age  $\geq 1$  month up to 17  $\mu\text{mol/L}$  (up to 1.0 mg/dL)

Reference range study with 500 well-characterized human serum samples:<sup>10</sup>

Males up to 24  $\mu\text{mol/L}$  (up to 1.4 mg/dL)

Females up to 15  $\mu\text{mol/L}$  (up to 0.9 mg/dL)

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term<sup>11</sup>

Age of newborn:

24 hours  $\geq 137 \mu\text{mol/L}^{b)}$  ( $\geq 8.0 \text{ mg/dL}^{b)}$ )

48 hours  $\geq 222 \mu\text{mol/L}^{b)}$  ( $\geq 13.0 \text{ mg/dL}^{b)}$ )

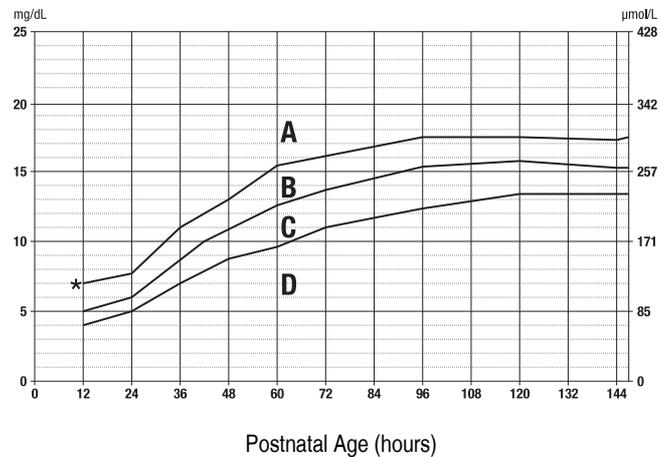
84 hours  $\geq 290 \mu\text{mol/L}^{b)}$  ( $\geq 17.0 \text{ mg/dL}^{b)}$ )

b) 95<sup>th</sup> percentile

Levels  $> 95^{\text{th}}$  percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

**Nomogram for designation of risk in 2840 well newborns<sup>11</sup>**

Serum Bilirubin



\* 95<sup>th</sup> 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 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
	$\mu\text{mol/L (mg/dL)}$	$\mu\text{mol/L (mg/dL)}$	%
Control level 1	15.4 (0.901)	0.3 (0.018)	2.1
Control level 2	52.8 (3.09)	0.3 (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
Intermediate precision	Mean	SD	CV
	$\mu\text{mol/L (mg/dL)}$	$\mu\text{mol/L (mg/dL)}$	%
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 corresponding reagent (x).

Sample size (n) = 64

Passing/Bablok <sup>12</sup>	Linear regression
$y = 0.995x + 0.734 \mu\text{mol/L}$	$y = 0.993x + 1.20 \mu\text{mol/L}$
$r = 0.990$	$r = 1.00$

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/Bablok <sup>12</sup>	Linear regression
$y = 0.962x + 1.55 \mu\text{mol/L}$	$y = 0.936x + 3.01 \mu\text{mol/L}$
$r = 0.981$	$r = 1.00$

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

**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.

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

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

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