

**Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
05795419 190	Bilirubin Total Gen.3 (600 tests)	System-ID 05 7483 9 <b>cobas c 701/702</b>
10759350 360	Calibrator f.a.s. (12 x 3 mL)	Code 401
12149435 160	Precinorm U plus (10 x 3 mL)	Code 300
12149443 160	Precipath U plus (10 x 3 mL)	Code 301
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3

**English**

For use in the USA only

**System information****3BILT:** ACN 8297**3SBIL:** ACN 8296 (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>

Measurement of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder blockage.

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 diazo method

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: 50 mmol/L; detergent; stabilizers; pH 1.0**R3** 3,5-dichlorophenyl diazonium salt:  $\geq 1.35$  mmol/L**(STAT R2)**

R1 is in position B and R3 (STAT 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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.

**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: 1-800-428-2336

**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, K<sub>2</sub>-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.

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability: a),3	1 day at 20-25 °C
	7 days at 4-8 °C
	6 months at -20 °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 <sub>2</sub> O)		
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 <sub>2</sub> O 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: This method has been 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**

**cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L × 0.0585 = mg/dL
	mg/dL × 10 = mg/L
	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:<sup>5</sup> 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 ± 0.10 mg/dL (1.7 µmol/L) of initial values of samples ≤ 1.0 mg/dL (17 µmol/L) and ± 10 % of samples > 1.0 mg/dL.

Hemolysis in neonates:<sup>5</sup> No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/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 a concentration of 0.12 mmol/L (3 mg/dL).

Cyanokit (Hydroxocobalamin) may cause false 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 **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

**3BILT**, ACN 8297

0.15-32.2 mg/dL (2.5-550 µmol/L)

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

**3SBIL**, ACN 8296

0.15-35.1 mg/dL (2.5-600 µmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun functions 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 = 0.10 mg/dL (1.7 µmol/L)

Limit of Detection = 0.15 mg/dL (2.5 µmol/L)

Limit of Quantitation = 0.15 mg/dL (2.5 µmol/L)

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 between-run coefficient of variation of  $\leq 20$  %. It has been determined using low concentration bilirubin samples.

#### Expected values

Adults<sup>9</sup> up to 1.2 mg/dL (up to 21 µmol/L)

Children with age  $\geq 1$  month<sup>9</sup> up to 1.0 mg/dL (up to 17 µmol/L)

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term<sup>10</sup>

Age of newborn:

24 hours  $\geq 8.0$  mg/dL<sup>b</sup>  $\geq 137$  µmol/L<sup>b</sup>

48 hours  $\geq 13.0$  mg/dL<sup>b</sup>  $\geq 222$  µmol/L<sup>b</sup>

84 hours  $\geq 17.0$  mg/dL<sup>b</sup>  $\geq 290$  µmol/L<sup>b</sup>

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. Roche has not evaluated reference ranges in a pediatric population.

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 mg/dL (µmol/L)	SD mg/dL (µmol/L)	CV %
Control level 1	0.92 (15.7)	0.01 (0.2)	1.2
Control level 2	3.11 (53.1)	0.02 (0.3)	0.6
Human serum A	0.53 (9.1)	0.01 (0.2)	2.5
Human serum B	18.1 (310)	0.06 (1)	0.4
Human serum C	26.9 (460)	0.2 (3)	0.7

Intermediate precision	Mean mg/dL (µmol/L)	SD mg/dL (µmol/L)	CV %
Control level 1	0.90 (15.4)	0.02 (0.3)	2.1
Control level 2	3.09 (52.8)	0.03 (0.5)	0.8
Human serum A	0.51 (8.7)	0.02 (0.3)	3.3
Human serum B	17.66 (302.0)	0.14 (2.4)	0.8
Human serum C	31.82 (544.1)	0.18 (3.1)	0.6

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

#### Method comparison

Total bilirubin values for human serum samples of adults obtained on a **cobas c 501** analyzer (y) using the Roche Bilirubin Total Gen.3 reagent were compared with those determined using the corresponding reagent on a **cobas c 701** analyzer (x).  
Sample size (n) = 61

Passing/Bablok<sup>11</sup>

$y = 0.994x - 0.004$  mg/dL

$\tau = 0.988$

Linear regression

$y = 0.993x - 0.001$  mg/dL

$r = 1.00$

The sample concentrations were between 0.24 and 30.4 mg/dL (4.1 and 519.3 µmol/L).

Total bilirubin values for human serum samples of newborns obtained on a **cobas c 501** analyzer (y) using the Roche Bilirubin Total Gen.3 reagent were compared with those determined using the Roche Bilirubin Total Special reagent on the same analyzer (x).  
Sample size (n) = 113

Passing/Bablok<sup>11</sup>

$y = 0.957x + 0.154$  mg/dL

$\tau = 0.973$

Linear regression

$y = 0.929x + 0.221$  mg/dL

$r = 1.00$

The sample concentrations were between 0.21 and 29.21 mg/dL (3.6 and 499.5 µmol/L).




#### References

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**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](http://dialog. Roche.com) for definition of symbols used):

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

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
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