BIL-D

cobas®

Bilirubin Direct

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20737496 322	Bilirubin Direct (350 tests)	System-ID 07 3749 6	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
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	

English

System information

For **cobas c** 311/501 analyzers:

BILDF: ACN 293

For **cobas c** 502 analyzer:

BILDF: ACN 8293

Intended use

In vitro test for the quantitative determination of direct (conjugated) bilirubin in human serum and plasma 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

Diazo method.[2]

Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with diazotized sulfanilic acid in acid buffer to form the red-colored azobilirubin.

sulfanilic acid + $NaNO_2$ ⟶ diazotized sulfanilic acid

bilirubin + diazotized sulfanilic acid ⟶ azobilirubin

The color intensity is proportional to the concentration of direct bilirubin in the sample and is determined by monitoring the increase in absorbance.

Reagents - working solutions

R1 Sulfanilic acid: 35 mmol/L; HEDTA: 4.0 mmol/L; oxalic acid: 40 mmol/L, pH 1.2

R2 Sodium nitrite: 3.9 mmol/L; pH 6.0

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:

sulphanilic acid

EUH 208 May produce an allergic reaction.

Warning

H290 May be corrosive to metals.

Prevention:

P234 Keep only in original container.

Response:

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

BIL-D

Shelf life at 15-25 °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 (free from hemolysis and lipemia): The specimen of choice is serum.
Plasma (free from hemolysis and lipemia): Li-heparin 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,3,4]	2 days 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

BIL-D

Bilirubin Direct

Wavelength (sub/main)	800/546 nm	
Reaction direction	Increase	
Units	µmol/L (mg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	54 µL	39 µL
R2	18 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	9 µL	–	–
Decreased	4.5 µL	–	–
Increased	9 µL	–	–

cobas c 501 **test definition**

Assay type	2-Point End
Reaction time / Assay points	10 / 10-25
Wavelength (sub/main)	800/546 nm
Reaction direction	Increase
Units	µmol/L (mg/dL, mg/L)
Reagent pipetting	Diluent (H_2O)
R1	54 µL 39 µL
R2	18 µL 20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	9 µL	–	–
Decreased	4.5 µL	–	–
Increased	9 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End
Reaction time / Assay points	10 / 10-25
Wavelength (sub/main)	800/546 nm
Reaction direction	Increase
Units	µmol/L (mg/dL, mg/L)
Reagent pipetting	Diluent (H_2O)
R1	54 µL 39 µL
R2	18 µL 20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	9 µL	–	–
Decreased	4.5 µL	–	–
Increased	18 µL	–	–

Calibration

Use a K factor. The K factor is 38200 µmol/L or 2235 mg/dL for the **cobas c** 311 analyzer and 34590 µmol/L or 2024 mg/dL for the **cobas c** 501/502 analyzers if reporting to two decimal places.

Calibrator	S1: H_2O
Calibration mode	Linear
Calibration frequency	Blank calibration - after reagent lot change - as required following quality control procedures

Traceability: This method has been standardized against the manual test performance using the Jendrassik Grof method.[5]

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 x 0.0585 = mg/dL
	mg/dL x 10 = mg/L
	mg/dL x 17.1 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a direct bilirubin concentration of 5.1 µmol/L (0.3 mg/dL).

Hemolysis:[6] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 15.6 µmol/L or 25 mg/dL).

Lipemia (Intralipid):[6] No significant interference up to an L index of 35. 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.[7,8]

Exception: Ascorbic acid, Intralipid (2000 mg/L) and rifampicin cause artificially high bilirubin results and phenylbutazone causes artificially low bilirubin results.

Samples containing indocyanine green must not be measured.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

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

2-430 µmol/L (0.1-25.2 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

Lower detection limit of the test

2 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[1]

Serum	µmol/L	mg/dL
Preterm infants: 1-6 days[10]	< 10*	< 0.6*
Infants > 1 month and adults	0-3.4	0-0.2

*The upper limit of 10 µmol/L (0.6 mg/dL) has been cited in the literature, although not ensured with internal data.

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

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*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	10.6 (0.620)	0.2 (0.012)	1.6
Precipath U	40.1 (2.35)	0.3 (0.02)	0.8
Human serum 1	2.97 (0.174)	0.09 (0.005)	3.1
Human serum 2	46.3 (2.71)	0.2 (0.01)	0.4
Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	10.0 (0.585)	0.3 (0.018)	3.1
Precipath U	40.2 (2.35)	1.2 (0.07)	3.0
Human serum 3	2.94 (0.172)	0.14 (0.008)	4.7
Human serum 4	44.6 (2.61)	0.8 (0.05)	1.7

Method comparison

A method comparison of the Roche BIL-D reagent (ACN 293) obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) against the D-BIL reagent obtained on a Roche/Hitachi 917 analyzer (x) using human serum samples gave the following results:

Sample size (n) = 84

Passing/Bablok[11]	Linear regression
y = 1.017x - 2.07 µmol/L	y = 1.055x - 3.01 µmol/L
τ = 0.929	r = 0.997

The sample concentrations were between 3.83 and 162 µmol/L (0.224 and 9.47 mg/dL).

References

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5 Jendrassik L, Grof P. Vereinfachte photometrische Methode zur Bestimmung des Bilirubins. Biochem Z 1938;297:81-89.

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7 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

8 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.

9 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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11 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

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www.roche.com