

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
20737496 322	Bilirubin Direct (350 tests)	System-ID 07 3749 6 COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 360	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6
12149435 160	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7
12149443 160	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7

English**System information**

Test BIL-D, test ID 0-249 on COBAS INTEGRA 400 plus systems;
Test ID 0-049 on COBAS INTEGRA 800 systems.

Intended use

In vitro test for the quantitative determination of direct (conjugated) bilirubin in human serum and plasma on COBAS INTEGRA systems.

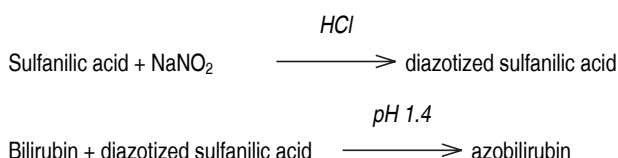
Summary¹

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 principle

Diazo method²

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



The color intensity is proportional to the concentration of direct bilirubin in the sample and is determined by monitoring the increase in absorbance at 552 nm.

Reagents - working solutions

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

R2 Sodium nitrite: 3.9 mmol/L; pH 6.0

R1 is in position A and R2 is in position B.

Precautions and warnings

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

For USA: For prescription use only.

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: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

Shelf life at 15-25 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus systems

On-board in use at 10-15 °C 8 weeks

COBAS INTEGRA 800 systems

On-board in use at 8 °C 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. Do not use other anticoagulants.

Protect specimens from exposure to light.

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.

Stability:^{a),3} 2 days 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

Centrifuge samples containing precipitates before performing the assay.

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-R2-S
Reaction direction	Increase
Wavelength A/B	552/652 nm
Calc. first/last	T ₀ /44
Unit	mg/dL

Pipetting parameters

		Diluent (H ₂ O)
R1	54 µL	24 µL
R2	18 µL	24 µL
Sample	9 µL	11 µL
Total volume	140 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-R2-S
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	T ₀ /35
Unit	mg/dL

Pipetting parameters

		Diluent (H ₂ O)
R1	54 µL	24 µL
R2	18 µL	24 µL
Sample	9 µL	11 µL
Total volume	140 µL	

Calibration

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

Traceability: This method has been standardized against the Doumas reference method.

Quality control

Reference range	Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U plus 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 × 10 = mg/L
	mg/dL × 17.1 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Hemolysis:⁴ No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6.2 µmol/L or 10 mg/dL).

Lipemia (Intralipid):⁴ No significant interference up to an L index of 270. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Therapeutic drug interference was tested according to the recommendations of the VDGH^{b)}. No interferences were found.

Samples containing indocyanine green must not be measured.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.⁵

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.

b) Verband der Diagnostica und Diagnostica Geräte Hersteller. Refer to section A of the Method Manual for a list of drugs tested and their concentrations.

Limits and ranges**Measuring range**

0.10-25 mg/dL (1.71-427.5 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

Lower detection limit of the test:

0.10 mg/dL (1.71 µmol/L)

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 values¹

Serum 0-0.2 mg/dL (0-3.4 µmol/L)

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.

BIL-D

Bilirubin Direct

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 20) and intermediate precision (n = 20). The following results were obtained:

	Level 1	Level 2
Mean	0.4 mg/dL	1.2 mg/dL
CV repeatability	1.7 %	0.53 %
CV intermediate precision	1.9 %	1.2 %

Method comparison

Direct bilirubin values for human serum and plasma samples obtained on a COBAS INTEGRA 800 analyzer with the COBAS INTEGRA Bilirubin Direct reagent (y) were compared with those determined using commercially available reagents for direct bilirubin on a Roche/Hitachi 917 analyzer (x). Sample size (n) = 41

Passing/Bablok ⁶	Linear regression
$y = 0.996x - 0.199$	$y = 1.011x - 0.314$
$r = 0.961$	$r = 0.997$
SD (md 95) = 0.406	$Sy.x = 0.223$




The sample concentrations were between 0.270 and 19.553 mg/dL (46.17 and 334.4 µmol/L).

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 Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937;119:481-490.
- 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 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 5 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 6 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.

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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