Bilirubin Direct

Order information

COBAS INTEGRA Bilirubin Direct	350 Tests	Cat. No. **20737496** 322 System-ID 07 3749 6
Calibrator f.a.s.	12 × 3 mL	Cat. No. **10759350** 190
Calibrator f.a.s. (for USA)	12 × 3 mL	Cat. No. **10759350** 360 System-ID 07 3718 6
Precinorm U[a]	20 × 5 mL	Cat. No. **10171743** 122 System-ID 07 7997 0
Precipath U	20 × 5 mL	Cat. No. **10171778** 122 System-ID 07 7998 9
Precinorm U plus[a]	10 × 3 mL	Cat. No. **12149435** 122
Precinorm U plus[a] (for USA)	10 × 3 mL	Cat. No. **12149435** 160 System-ID 07 7999 7
Precipath U plus	10 × 3 mL	Cat. No. **12149443** 122
Precipath U plus (for USA)	10 × 3 mL	Cat. No. **12149443** 160 System-ID 07 8000 6
Precibil	4 × 2 mL	Cat. No. **10158046** 122 System-ID 07 6604 6

a) for COBAS INTEGRA 400/400 plus systems only

● Indicates analyzer(s) on which **cobas c** pack can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 800
●	●

System information

COBAS INTEGRA Bilirubin Direct (BIL-D)
Test BIL-D, test ID 0-249 on COBAS INTEGRA 400/400 plus systems; test ID 0-049 on COBAS INTEGRA 800 systems.

Intended use

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

$$\text{sulfanilic acid} + NaNO_2 \xrightarrow{HCl} \text{diazotized sulfanilic acid}$$

$$\text{bilirubin+ diazotized sulfanilic acid} \xrightarrow{pH\ 1.4} \text{azobilirubin}$$

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

Components	Concentrations R1	R2	Test	
Sulfanilic acid	35		13.5	mmol/L
Oxalic acid	40		15.4	mmol/L
HEDTA	4.0		1.5	mmol/L
Sodium nitrite		3.9	0.5	mmol/L
pH	1.2	6.0	1.4	

Precautions and warnings

Pay attention to all precautions and warnings listed in this Method Manual, Chapter 1, Introduction.
FOR US CUSTOMERS: CORROSIVE
In case of contact, flush the affected area with copious amounts of water. Get immediate medical attention if the reagent comes into contact with eyes, or if ingested.

Reagent handling

Ready for use.

Storage and stability

Shelf life at 15 to 25 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400/400 plus analyzers	
On-board in use at 10 to 15 °C	8 weeks
COBAS INTEGRA 800 analyzers	
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:[b,3] 2 days at 15-25 °C
7 days at 2-8 °C
6 months at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.
b) If care is taken to prevent exposure to light

Materials provided
See "Reagents - working solutions" section for reagents.

Assay
For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Application for serum and plasma

COBAS INTEGRA 400/400 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	µmol/L

Pipetting parameters

		Diluent (H_2O)
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	µmol/L

Pipetting parameters

		Diluent (H_2O)
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 manual test performance using the Jendrassik Grof method. In the USA, this method was standardized against the Doumas reference method.

Quality control

Reference range	*COBAS INTEGRA 400/400 plus systems:* Precinorm U or Precinorm U plus *COBAS INTEGRA 800 systems:* Precipath U or Precipath U plus, diluted 1 + 2 with distilled water after reconstitution.
Pathological range	Precipath U or Precipath U plus or Precibil
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. Other suitable control material can be used in addition.

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 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 400/400 plus/800 analyzers).

Conversion factor: µmol/L × 0.0585 = mg/dL

Limitations - interference[4]
Criterion: Recovery within ± 10 % of initial value

Serum, plasma

Hemolysis	No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6 µmol/L or 10 mg/dL).
Lipemia (Intralipid)	No significant interference up to an L index of 270. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Drugs	Therapeutic drug interference was tested according to the recommendations of the VDGH[c]. No interference was found.
Other	In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

c) Verband der Diagnostica und Diagnostica Geräte Hersteller

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 Method Manual, Introduction, Extra Wash Cycles for further instructions. **Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

Limits and ranges

Measuring range

1.7-430 µmol/L (0.10-25 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 by the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Lower detection limit of the test:
1.7 µmol/L (0.10 mg/dL)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample + 3 SD, within-run precision, n = 30).

Expected values

Serum

Preterm infants (1-6 days)[5]	< 10 µmol/L (< 0.6 mg/dL)*
Infants >1 month and adults[1]	0-3.4 µmol/L (0-0.2 mg/dL)

*The upper limit of 10 µmol/L (0.6 mg/dL) direct bilirubin for preterm infants 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 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 (within-run n = 20, between-run n = 20). The following results were obtained:

	Level 1	Level 2
Mean	6.1 µmol/L (0.4 mg/dL)	20.1 µmol/L (1.2 mg/dL)
CV within-run	1.7 %	0.53 %
CV between-run	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 to those determined with Roche/Hitachi reagent for direct bilirubin on a Roche/Hitachi 717 analyzer *(x)*.

Roche/Hitachi 717 analyzer	Sample size (n) = 49
Passing/Bablok[6]	Linear regression
$y = 1.030x - 2.018$ µmol/L	$y = 1.088x - 3.839$ µmol/L
$\tau = 0.913$	r = 0.996
SD (md 95) = 5.581	Sy.x = 4.154

The sample concentrations were between 0.885 and 185.05 µmol/L (0.052 and 10.83 mg/dL).

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. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes; Pre-analytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag 1996.
4. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
5. Soldin JS, Brugnara C, Wong C. Pediatric Reference Intervals. AACC Press, 5th ed., 2005.
6. Passing H, Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

Roche Diagnostics GmbH, D-68298 Mannheim
Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

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