# BILTS

**Total Bilirubin Special**

cobas®

**Substrates**

**Order information**

| REF | CONTENT | | Analyzers on which **cobas c** pack can be used |
|---|---|---|---|
| **03261638** 190 | Total Bilirubin Special (250 tests) | System-ID 07 6721 2 | COBAS INTEGRA 400 plus<br>COBAS INTEGRA 800 |
| **10759350** 190 | Calibrator f.a.s. (12 x 3 mL) | System-ID 07 3718 6 | |
| **12149435** 122 | Precinorm U plus (10 x 3 mL) | System-ID 07 7999 7 | |
| **12149443** 122 | Precipath U plus (10 x 3 mL) | System-ID 07 8000 6 | |
| **10171743** 122 | Precinorm U (20 x 5 mL) | System-ID 07 7997 0 | |
| **10171735** 122 | Precinorm U (4 x 5 mL) | System-ID 07 7997 0 | |
| **10171778** 122 | Precipath U (20 x 5 mL) | System-ID 07 7998 9 | |
| **10171760** 122 | Precipath U (4 x 5 mL) | System-ID 07 7998 9 | |
| **05117003** 190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | System-ID 07 7469 3 | |
| **05947626** 190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | System-ID 07 7469 3 | |
| **05117216** 190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | System-ID 07 7470 7 | |
| **05947774** 190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | System-ID 07 7470 7 | |
| **10158046** 122 | Precibil (4 x 2 mL) | System-ID 07 6604 6 | |

**English**

**System information**

Test BILTS, test ID 0-985

**Intended use**

In vitro test for the quantitative determination of total bilirubin in serum and plasma of adults and neonates 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]

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a diazonium ion in a strongly acidic medium (pH 1-2) to form azobilirubin.

Bilirubin + diazonium ion $\xrightarrow{\text{acid}}$ azobilirubin

The intensity of the color of the azobilirubin produced is proportional to the total bilirubin concentration and can be measured photometrically.

**Reagents - working solutions**

**R1** Sulfamic acid ($H_3NO_3S$): 110 mmol/L; sodium acetate buffer ($C_2H_3NaO_2$ ): 85 mmol/L; surfactant; solubilizer

**SR** Diazonium ion: 3 mmol/L; HCl: 100 mmol/L

R1 is in position B, and SR is in position C.

**Precautions and warnings**

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

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

**Hazardous components:** Betaines, C12-14-alkyldimethyl

Danger

| | |
|---|---|
| H290 | May be corrosive to metals. |
| H314 | Causes severe skin burns and eye damage. |

**Prevention:**

| | |
|---|---|
| P234 | Keep only in original container. |
| P264 | Wash skin thoroughly after handling. |
| P280 | Wear protective gloves/ protective clothing/ eye protection/ face protection. |

**Response:**

| | |
|---|---|
| P301 + P330 + P331 | IF SWALLOWED: rinse mouth. Do NOT induce vomiting. |
| P303 + P361 + P353 | IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. |
| P304 + P340 | IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. |
| P305 + P351 + P338 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| P310 | Immediately call a POISON CENTER or doctor/physician. |
| P363 | Wash contaminated clothing before reuse. |
| P390 | Absorb spillage to prevent material damage. |

**Storage:**

| | |
|---|---|
| P405 | Store locked up. |
| P406 | Store in corrosive resistant stainless steel container with a resistant inner liner. |

**Disposal:**

| | |
|---|---|
| P501 | Dispose of contents/ container to an approved waste disposal plant. |

Contact phone: all countries: +49-621-7590

**Reagent handling**

Ready for use

# BILTS

**Total Bilirubin Special**

cobas®

**Substrates**

### Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C | See expiration date on **cobas c** pack label |
| COBAS INTEGRA 400 plus system | |
| On-board in use at 10-15 °C | 5 weeks |
| COBAS INTEGRA 800 system | |
| On-board in use at 8 °C | 5 weeks |

Store reagent protected from light.

Any discoloration or particle formation that may occur does not affect reagent performance.

### 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: Potassium EDTA and lithium heparin are the only acceptable anticoagulants.
Do not use cord blood samples.

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] | 1 day at 15-25 °C<br>7 days at 2-8 °C<br>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.

### 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-S-SR |
| Reaction direction | Increase |
| Wavelength A/B | 552/629 nm |
| Calc. first/last | 33/50 |
| Unit | µmol/L |

#### Pipetting parameters

| | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 120 µL | 0 µL |
| Sample | 2 µL | 5 µL |
| SR | 32 µL | 0 µL |
| Total volume | 159 µL | |

#### COBAS INTEGRA 800 test definition

| | |
|---|---|
| Measuring mode | Absorbance |
| Abs. calculation mode | Endpoint |
| Reaction mode | R1-S-SR |
| Reaction direction | Increase |
| Wavelength A/B | 552/629 nm |
| Calc. first/last | 44/74 |
| Unit | µmol/L |

#### Pipetting parameters

| | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 120 µL | 0 µL |
| Sample | 2 µL | 5 µL |
| SR | 32 µL | 0 µL |
| Total volume | 159 µL | |

#### Calibration

| | |
|---|---|
| Calibrator | C.f.a.s.<br>Use deionized water as zero calibrator. |
| Calibration mode | Linear regression |
| Calibration replicate | Duplicate recommended |
| Calibration interval | Each **cobas c** pack, every 7 days, and as required following quality control procedures |

Traceability: The method was standardized against the Doumas method.

#### Quality control

| | |
|---|---|
| Reference range | Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1 |
| Pathological range | Precipath U, Precipath U plus, Precibil 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 factor: µmol/L × 0.0585 = mg/dL

### Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Hemolysis:[4] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[4] No significant interference up to an L index of 1400. 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.[5,6]Exceptions: Ascorbic acid at 30 mg/dL causes artificially decreased total bilirubin values.
Hydroxocobalamin (Cyanokit) may cause false-low results.

Anticoagulants: Underfilled heparinized tubes may cause elevated results. Verify all neonatal T-Bilirubin results in heparin samples in the therapeutic decision range.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[7]

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.

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 (0.099-25.2 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:4 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 4.

**Lower limits of measurement**

Lower detection limit of the test:
1.7 µmol/L (0.099 mg/dL)

The lower detection limit represents the lowest measurable bilirubin concentration that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 21).

**Expected values**

| | | |
|---|---|---|
| Adults[8] | up to 21 µmol/L | (up to 1.2 mg/dL) |
| Children with age ≥ 1 month[8] | up to 17 µmol/L | (up to 1.0 mg/dL) |

Reference range study with 500 well-characterized human serum samples[9]

| | | |
|---|---|---|
| Males | up to 24 µmol/L | (up to 1.4 mg/dL) |
| Females | up to 15 µmol/L | (up to 0.9 mg/dL) |

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[10]

Age of newborn:

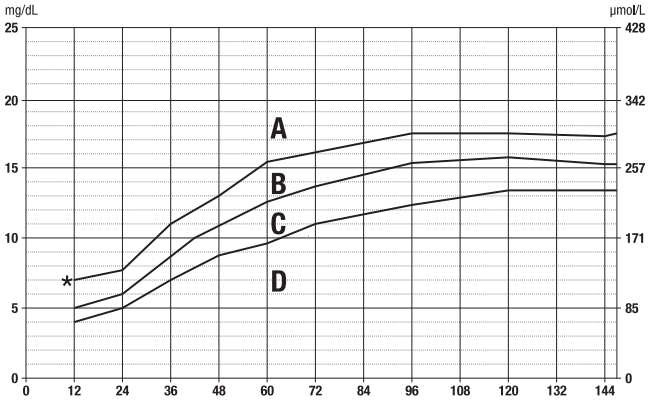
| | | |
|---|---|---|
| 24 hours | ≥ 137 µmol/L[b)] | (≥ 8.0 mg/dL[b)]) |
| 48 hours | ≥ 222 µmol/L[b)] | (≥ 13.0 mg/dL[b)]) |
| 84 hours | ≥ 290 µmol/L[b)] | (≥ 17.0 mg/dL[b)]) |

b) 95th percentile

Levels > 95th 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[10]

Serum Bilirubin




***** 95th percentile

**A** High risk zone

**B** High intermediate risk zone

**C** Low 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**

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* | Level 1 | Level 2 |
|---|---|---|
| Mean | 15.8 µmol/L (0.924 mg/dL) | 54.0 µmol/L (3.16 mg/dL) |
| CV | 2.4 % | 1.4 % |

| *Intermediate precision* | Level 1 | Level 2 |
|---|---|---|
| Mean | 14.7 µmol/L (0.860 mg/dL) | 47.2 µmol/L (2.76 mg/dL) |
| CV | 4.1 % | 2.2 % |

**Method comparison**

Total bilirubin values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Total Bilirubin Special reagent (y) were compared with those determined using reagents for total bilirubin on a COBAS INTEGRA 700 analyzer (x) and on a Roche/Hitachi 917 analyzer (x) . Samples were measured in duplicate. Sample size (n) represents all replicates.

The sample concentrations were between 1.46 and 525 µmol/L (0.085 and 30.7 mg/dL).

| **COBAS INTEGRA 700 analyzer** | Sample size (n) = 62 |
|---|---|
| Passing/Bablok[11] | Linear regression |
| y = 1.046x - 2.82 µmol/L | y = 1.067x - 4.07 µmol/L |
| r = 0.999 | $r_s$ = 0.960 |

| **Roche/Hitachi 917 analyzer** | Sample size (n) = 73 |
|---|---|
| Passing/Bablok[11] | Linear regression |
| y = 1.012x + 0.258 µmol/L | y = 0.999x + 0.895 µmol/L |
| r = 1.00 | $r_s$ = 0.968 |

**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 Wahlefeld AW, Herz G, Bernt E. Modification of the method for a simple, reliable determination of total bilirubin in serum. Scand J Clin Lab Invest 1972;29:Supplement 126:Abstract 11.12.

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

6 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.

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

8 Thomas L, ed. Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die Medizinische Diagnostik, 7th ed.: TH-Books Verlagsgesellschaft 2007:259-273.

9 Löhr B, El-Samalouti V, Junge W, et al. Reference Range Study for Various Parameters on Roche Clinical Chemistry Analyzers. Clin Lab 2009;55:465-471.

10 Subcommittee on Hyperbilirubinemia. Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation. Pediatrics 2004;114:297-316.

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 |



CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche