Application Sheet

Laboratory Name
Test Name: Direct Bilirubin

System Information

For **cobas c** 311/501 analyzers:
DBILI: ACN 006

Intended use

For the quantitative determination of direct bilirubin in serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Bilirubin is an organic compound formed by the reticuloendothelial system during the normal and abnormal destruction of red blood cells. Measurements of bilirubin are used in the diagnosis of liver disease, in the detection of hemolytic anemia, and to evaluate degrees of jaundice.
Since the introduction of the diazo method for bilirubin determination by Ehrlich in 1883[1], several modifications have been proposed to enhance the reaction. The Evelyn-Malloy method[2] employs methanol to catalyze the azo-coupling reaction of the indirect bilirubin, as well as to keep the azobilirubin in solution. A serious disadvantage of this method lies in the fact that protein may be precipitated by the methanol solution to yield falsely lowered results.
In 1938, Jendrassik and Grof[3] presented an assay that gave reliable results. The advantages of this method over the Evelyn-Malloy procedure include greater precision, reduction of interference by pigments such as hemoglobin and serum contents (e.g., urobilinogen, uric acid and carotenoids), and reduction of turbidity produced by alcohol denaturation of proteins.
The Roche Diagnostics Direct Bilirubin method, based on the Jendrassik-Grof procedure, is standardized against the manual direct bilirubin procedure of Lo and Wu.[4]

Test principle

Acidified sodium nitrite produces nitrous acid, which reacts with sulfanilic acid (in acidic solution) to form a diazonium salt. The diazotized sulfanilic acid then reacts with bilirubin to form isomers of azobilirubin. In the direct bilirubin assay, only conjugated bilirubin is converted by the diazotized sulfanilic acid. The intensity of the red color of azobilirubin is measured photometrically and is proportional to the direct (conjugated) bilirubin concentration.

Reagents - working solutions

R1	Hydrochloric acid: 0.05 mol/L
R2	Sulfanilic acid: 25.7 mmol/L; hydrochloric acid: 0.7 mol/L; sodium nitrite: 2.7 mmol/L; sodium bicarbonate: 13.9 mmol/L

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: 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.

Danger

H314	Causes severe skin burns and eye damage.
Prevention:	
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): Take off immediately all contaminated clothing. Rinse skin with water/shower.
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.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling primarily follows EU GHS guidance.
Contact phone: 1-800-428-2336

Reagent handling

For reagent handling instructions, refer to the **cobas c** D Bili transfer instruction sheet contained in the reagent packaging.

Storage and stability

Unopened kit components: up to the expiration date at 15-25 °C
On-board in use and refrigerated on the analyzer: 14 days

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: Collect serum using standard sampling tubes.
Use non-hemolyzed serum.
Plasma: Use Li-heparin plasma. Do not use other anticoagulants.
Use non-hemolyzed 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),5]	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

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section
General laboratory equipment
In addition, other suitable control material can be used.

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 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-36		
Wavelength (sub/main)	660/570 nm		
Reaction direction	Increase		
Units	mg/dL		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	-	
R2	20 µL	-	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (H_2O)
Normal	6 µL	-	-
Decreased	6 µL	-	-
Increased	6 µL	-	-

Calibration

Use a K factor. The K factor is 237 if reporting to one decimal place or 2372 if reporting to two decimal places.

Calibrators	S1: H_2O
Calibration mode	Linear
Calibration frequency	Blank calibration • every 24 hours • after cassette change • after reagent lot change • as required following quality control procedures

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

Quality control

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy

Refer to Brown Clinic Quatliy Control Specialty and Subspecialty Policy

---

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 0.2 mg/dL (3.5 µmol/L).
Hemolysis:[6] No significant interference up to an H index of 30 (approximate hemoglobin concentration: 18.62 µmol/L or 30 mg/dL).
Lipemia (Intralipid):[6] No significant interference up to an L index of 100. 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 at the therapeutic drug level.
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

0.2-10.0 mg/dL (3.5-171 µmol/L)
Determine samples with bilirubin concentrations > 10 mg/dL (> 171 µmol/L) by manually diluting samples with low normal serum (e.g. 1+1). Multiply the result by the appropriate dilution factor (e.g. 2) and subtract the value of the low normal serum. Do not use water, saline or commercial albumin preparations to dilute patient samples.
Do not report results above 10 mg/dL (171 µmol/L) unless the sample has been manually pre-diluted.

Lower limits of measurement

Lower detection limit of the test

0.1 mg/dL (2 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 values[10]

Serum/plasma	0.0-0.3 mg/dL (0.0-5.1 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

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

1 Ehrlich P. Charite Ann 1883:8;140.
2 Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937;119:481-490.
3 Jendrassik L, Grof P. Biochem J 297, 81-89 (1938).
4 Lo DH, Wu TW. Assessment of the fundamental accuracy of the Jendrassik-Gróf total and direct bilirubin assays. Clin Chem 1983;29:31-36.
5 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.
6 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
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.
10 Sherlock S. Liver Disease 1958 Churchill, London 1958.
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.

Alternative method

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: DBILI
Method Sheet Version: V13.0 English

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04924495 190	Direct Bilirubin (3 x 350 tests)	System-ID 07 6968 1	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
12149435 160	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 160	Precipath U plus (10 x 3 mL)	Code 301	
On request	Open/close tool		

Effective date

Effective date for this procedure:

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)^{cm} Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

REVIEW – REVISION SUMMARY DOCUMENTATION

Date: By: Revision Summary: