

BILIRUBIN (DIRECT)

BD 0480 CH

450 ml

SUMMARY OF TEST

Bilirubin, is produced from protoporphyrin IX by microsomal heme oxygenase. Daily bilirubin production in man averages 250 to 300 mg. After production, bilirubin is transported to the liver in association with albumin. Bilirubin is then rapidly taken up by hepatocytes by what is presumed to be a carrier-mediated active transport process across the sinusoidal membrane. Once inside the liver cells, bilirubin is tightly but reversibly bound to soluble proteins. Then it is rapidly conjugated with glucuronic acid to produce bilirubin mono- and diglucuronide, which are excreted into bile.

UNCONJUGATED HYPERBILIRUBINEMIA. The most commonly occurring form of unconjugated hyperbilirubinemia is that seen in newborns and referred to as physiological jaundice. All newborns have serum unconjugated bilirubin concentrations greater than values obtained in the healthy adult population. A common inherited disorder, Gilbert's syndrome, is a heterogeneous condition that is usually ascribed to decreased UDP-glucuronyltransferase activity but may also be caused by defects in membrane transport. Because bilirubin cannot be conjugated at the normal rate in patients with these disorders, the rate of excretion of bilirubin is significantly reduced, and the serum concentration of unconjugated bilirubin increases.

CONJUGATED HYPERBILIRUBINEMIA. In hepatobiliary diseases of various causes, bilirubin uptake, storage, and excretion are impaired to varying degrees. Thus, both conjugated and unconjugated bilirubin are retained in these disorders, and a wide range of abnormal serum concentrations of each form of bilirubin may be observed. When any portion of the biliary tree becomes blocked or abnormally permeable, biliary passage of bilirubin and of all other components of bile is retarded; thus, these substances are retained. As a result, plasma concentrations of conjugated bilirubin increase to abnormal values.

Numerous separation methods have been used to study the distribution and clinical importance of bilirubin and its metabolites in serum. These methods were severely limited by the fact that instability of the pigments made the identification and quantitation of the separated bilirubin species unreliable. The most widely used methods for bilirubin measurement are those based on the diazo reaction, which was first described in 1883 by Ehrlich. In this reaction, diazotized sulfanilic acid (the diazo reagent) reacts with bilirubin to produce two azodipyrroles, which are reddish purple at neutral pH and blue at low or high pH values. In 1916, Van den Bergh and Muller applied this reaction to the quantitation of bilirubin in serum and established that alcohol accelerated the diazotization reaction of unconjugated bilirubin. These investigators described the fraction of bilirubin that reacted with the diazo reagent in the absence of alcohol as the "direct" bilirubin fraction. They used the term "indirect" bilirubin for the difference between total bilirubin and the so-called "direct bilirubin" fraction. The diazo method originally described by Jendrassik and Grof in 1938 gives results for serum bilirubin that are reliable judged by the HPLC method. In the procedure, an aqueous solution of caffeine and sodium benzoate serves as the accelerator. In detailed studies using samples prepared by the addition of unconjugated bilirubin and authentic human diconjugated bilirubin to low-bilirubin pooled sera, Lo and Wu have shown that the modified Jendrassik-Grof total bilirubin assay detects unconjugated and conjugated bilirubin quantitatively. This method has acceptable transferability amongst laboratories and is currently the method of choice.

PRINCIPLE OF THE METHOD

Conjugated (direct) bilirubin reacts with diazotized sulfanilic acid to produce an intensely colored diazo dye (520-560 nm). The intensity of color of this dye in solution is proportional to the concentration of direct bilirubin.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A: 3 x 120 ml (liquid) blue cap

Composition: sodium chloride 0.26 M, EDTA 0.01 M.

Reagent B1: 1 x 90 ml (liquid) red cap

Composition: sulphaniilic acid 30 mM, hydrochloric acid 0.18 M.

Reagent B2: 1 x 15 ml (liquid) red cap

Composition: sodium nitrite 72 mM.

Store all components at 15-25°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution. Calibrator.

REAGENT PREPARATION

Reagent A, ready to use.

Reagent B, mix 30 parts of reagent B1 and 1 part of reagent B2. Mixed reagent must rest at least 30 minutes before use.

Stable 30 days at 2-8°C.

Caution: keep well refrigerated.

Stability of unmixed reagents:

up to expiration date on labels at 15-25°C.

Stability of unmixed reagents since first opening of vials:

≥ 60 days at 15-25°C -away from light sources-

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

Labelling Reagent A: Contains sulphaniilic acid. May produce an allergic reaction.

SPECIMEN

Serum, plasma.

Specimens should be protected from direct exposure to light. Samples stored at 2-8°C in the dark are stable up to 3 days and 1 month at -20°C.

TEST PROCEDURE

Wavelength:	546 nm (allowed 520 ÷ 560 nm)		
Lightpath:	1 cm		
Temperature:	25, 30 or 37°C		

dispense:	blank	calibrator	sample
reagent A	2 ml	2 ml	2 ml
water	200 µl	-	-
calibrator	-	200 µl	-
sample	-	-	200 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of calibrator (Ac₁) and samples (Ax₁) against reagent blank.

dispense:	blank	calibrator	sample
reagent B	500 µl	500 µl	500 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of calibrator (Ac₂) and samples (Ax₂) against reagent blank.

RESULTS CALCULATION

serum/plasma sample:

$$\text{bilirubin mg/dl} = \frac{Ax_2 - Ax_1}{Ac_2 - Ac_1} \times \text{calibrator value}$$

EXPECTED VALUES

adults: ≤ 0.20 mg/dl (≤ 3.4 µmol/l)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 20 mg/dl.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 0.17 mg/dl.

Interferences

no interference was observed by the presence of:
lipids ≤ 300 mg/dl
Hemoglobin interferes

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	0.734	0.005	0.70
sample 2	2.488	0.015	0.60

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	0.898	0.023	2.60
sample 2	2.355	0.065	2.80

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

$$\begin{aligned} \text{Bilirubin total Chema} &= x \\ \text{Bilirubin total competitor} &= y \\ n &= 111 \end{aligned}$$

$$y = 0.995x + 0.007 \text{ mg/dl} \quad r = 0.994$$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES








Jendrassik L., Grof P. - Biochemistry 297,81 (1938).

Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions