DIRECT BILIRUBIN FL

DD F125 CH	5 x 25 ml
DD F500 CH	10 x 50 ml

INTENDED USE

Reagent for quantitative in vitro determination of direct bilirubin in biological fluids.

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.

PRINCIPLE OF THE METHOD

Conjugated (direct) bilirubin reacts with diazotized 2,4-dichloroaniline in acidic solution to produce an intensely coloured red diazo compound (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.

BIL D R1 **F125: 4 x 25 ml (liquid) blue cap**
F500: 8 x 50 ml (liquid) blue cap

Composition: sodium chloride 0.26 M, EDTA 0.1 mM.

BIL D R2 **F125: 1 x 25 ml (liquid) red cap**
F500: 2 x 50 ml (liquid) red cap

Composition: EDTA 0.1 mM, diazotized 2,4-dichloroaniline 0.1 mM, hydrochloric acid 0.18 M.

Store all components at 2-8°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.

REAGENT PREPARATION

Use separate reagent ready to use.
Stability: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: preferably within 60 days at 2-8°C -away from light sources-.
Caution: keep well refrigerated.

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.

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 (BY CALIBRATOR)

Wavelenght: 546 nm (allowed 530 ÷ 560 nm)
Lightpath: 1 cm
Temperature: 25, 30 or 37°C

dispense:	blank	calibrator	sample
reagent R1	1 ml	1 ml	1 ml
water	50 µl	-	-
calibrator	-	50 µl	-
sample	-	-	50 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of calibrator (Ac_1) and samples (Ax_1) against reagent blank.

dispense:	blank	calibrator	sample
reagent R2	250 µl	250 µl	250 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of calibrator (Ac_2) and samples (Ax_2) against reagent blank.

TEST PROCEDURE (BY FACTOR)

Wavelenght: 546 nm
Lightpath: 1 cm
Temperature: 25, 30 or 37°C
Factor: 63.2

dispense:	blank	sample
reagent R1	1 ml	1 ml
water	50 µl	-
sample	-	50 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of samples (Ax_1) against reagent blank.

dispense:	blank	sample
reagent R2	250 µl	250 µl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of samples (Ax_2) against reagent blank.

RESULTS CALCULATION

Calibrated procedure:

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

Factored procedure:

$$\text{bilirubin mg/dl} = (Ax_2 - Ax_1) \times 63.2$$

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:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 13 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 0.039 mg/dl.

Interferences
No interference was observed by the presence of:

hemoglobin	≤ 50 mg/dl
lipids	≤ 500 mg/dl
ascorbic acid	≤ 30 mg/dl

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	0.719	0.003	0.44
sample 2	2.430	0.019	0.78

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	0.735	0.039	5.31
sample 2	2.456	0.105	4.26

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

Bilirubin direct Chema = x
Bilirubin direct competitor = y
n = 110

$y = 0.911x - 0.049$ mg/dl $r^2 = 0.995$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

Royden N., R. and A. di Pasqua - Clin. Chem. 570-578, 8 (1962).
J.A. Lott and B.T. Doumas - Clin. Chem. 641-647, 39 (1993).
Tietz Textbook of Clinical Chemistry, Fourth Edition, Burtis-Ashwood-Bruns (2006).

MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

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