Bilirubin Direct Kit

CliniQuant - FSR

DCA Method

Meril Diagnostics

For *in vitro* diagnostic use
Read this pack insert thoroughly before use

REF	Pack Size	R1 Bilirubin Direct Reagent	R2 Sodium Nitrite Reagent
DBLFSR-01	4 x 25ml	4 x 25ml	1 x 6ml

INTENDED USE

This reagent is intended for quantitative determination of bilirubin direct concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

Bilirubin a breakdown product of hemoglobin in the reticulo endothelial system is transported to the liver in association with albumin. In the liver, bilirubin is conjugated to glucuronic acid (mono and di glucuronides) to form conjugated bilirubin by the enzyme uridyldiphosphate glucuronyl transferase. This fraction of bilirubin is referred to as direct or conjugated bilirubin. Direct bilirubin is elevated in conditions causing hepatic obstruction, hepatitis, cirrhosis, several inherited enzyme deficiencies, and inherited defects in canalicular excretion.

PRINCIPLE OF THE METHOD

Bilirubin determination is generally based on the reaction of bilirubin with Dichloro aniline. In this method, direct (conjugated fractions) bilirubin couples with a Dichloro aniline in the presence of hydrochloric acid to form the colored compound azobilirubin. The increase in absorbance at 546 nm due to azobilirubin is proportional to the direct bilirubin concentration.

KIT COMPONENTS

Composition :

R1 - Bilirubin Direct Reagent : Sulfanilic Acid 6.24 g/l, 2,4 Dichloroaniline 0.22 g/l, HCl 10 ml/l

R2 - Sodium Nitrite Reagent : Sodium Nitrite 0.075 g/l

MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated

micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath, controls, saline.

REAGENT PREPARATION, STORAGE & STABILITY

Reagents are ready for use. Keep away from direct light sources.

Stability: unopened bottle up to expiration date on labels at +15 to +30°C.

Stability since first opening of bottle: preferable within 30 days at +15 to +30°C.

REAGENT DETERIORATION

Discard the working reagent if it fails to achieve assigned assay values of fresh control sera.

WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
2. Specimens should be considered infectious and handled appropriately.
3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma (heparinate only). Bilirubin in serum is stable for one month at -20°C or 7 days at 2-8 °C.

Programme Parameter for MERILYZER CliniQuant

Reading Mode	End Point
Factor	10
Filter 1 (nm)	546
Filter 2 (nm)	670
Temperature	37 °C
Volume (µl)	500
Delay Time (Sec)	5
Unit	mg/dl
Reaction Direction	Increase
Reference Low	0
Reference High	0.3
Linearity Limit	20

CE
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TEST PROCEDURE

Dispense	Blank	Test
Reagent 1	1ml	1 ml
Reagent 2	25 µl	25 µl
Distilled water	100 µl	-
Sample	-	100 µl
Mix, incubate for 1 min at 37°C. Read absorbance at 546/ 670 nm against reagent blank. Reading should be taken immediately after incubation is over (2 minutes maximum).		

RESULT CALCULATION

Direct Bilirubin mg/dl = Abs. of Test- Abs. of Blank x 10

SI conversion factor: 1 mg/dl x 17.1 = 1 µmol/l

EXPECTED VALUES

Direct Bilirubin:

Adults & Infants: 0 – 0.3 mg/dl OR 0 – 5.1 µmol/l

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

QUALITY CONTROL AND CALIBRATION

It is recommended to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

When using the recommended Calibrator (BioCal), calibrate the assay:

a. When using a new reagent or lot

b. When QC values are out of range

PERFORMANCE CHARACTERISTICS

1. Linearity

The linearity is up to 20 mg/dl or 342 µmol/l.

2. Sensitivity/ Limit of detection (LOD)

The limit of detection is 0.06 mg/dl.

The limit of quantification is 0.17 mg/dl.

3. Interferences

Gross hemolysis and/ or lipaemia may cause falsely low and/ or elevated results respectively.

4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	mg/dl	mg/dl	%
sample 1	0.53	0.01	2.43
sample 2	2.47	0.03	1.03

Inter-assay precision

	Mean	SD	CV
n = 20	mg/dl	mg/dl	%
sample 1	0.45	0.02	4.28
sample 2	2.46	0.05	1.91

5. Methods Comparison

Comparison was done between reference Direct Bilirubin Reagent and CliniQuant - FSR Direct Bilirubin Reagent (test).

N = 24 $y = 1.640x - 0.208$

$r^2 = 0.920$

LIMITATIONS

After 2 minutes, indirect bilirubin reacts slowly with diazotized dichloroaniline leading to over-estimated values.

WASTE DISPOSAL

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

REFERENCES

1. Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, W.B. Saunders Company, 1994, p. 1458 - 1470.

2. Data on file: Meril Diagnostics.

IFU/DBLFSR01/00

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Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged
EC REP	Authorized European Representative in the European Community		