QUADRAPED™

BILIRUBIN KIT

(Mod. Jendrassik and Grof's Method)

(For veterinary invitro diagnostic use only)

INTENDED USE

Bilirubin kit is used for the determination of Direct and Total Bilirubin in serum.

SUMMARY

Bilirubin is mainly formed from the heme portion of aged or damaged RBC'S. It then combines with albumin to form a complex, which is not water-soluble. This is referred to as indirect or unconjugated Bilirubin. In the liver this Bilirubin complex is combined with glucuronic acid into a water-soluble conjugate. This is referred to as conjugated or direct Bilirubin. Elevated levels of bilirubin are found in liver diseases (Hepatitis, cirrhosis), excessive hemolysis / destruction of RBC (hemolytic jaundice) obstruction of the biliary tract (obstructive jaundice) and in drug induced reactions. The differentiation between the direct and indirect bilirubin is important in diagnosing the cause of hyperbilirubinemia.

PRINCIPLE

Bilirubin reacts with diazotised sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin couples with the sulphanilic acid in the presence of a caffein-benzoate accelerator. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

Bilirubin + Diazotized Sulphanilic Acid ⟶ Azobilirubin Compound

EXPECTED VALUES

Species	Total Bilirubin(mg/dl)	Direct Bilirubin(mg/dl)
Dog	0 - 0.3	0 - 0.1
Cat	0 - 0.1	0 - 0.1
Cow	0 - 1.6	0 - 0.1
Horse	0.5 - 3.5	0 - 0.4
Pig	0 - 1.0	0 - 0.2
Sheep	0.1 - 0.5	0 - 0.1
Goat	0.1 - 0.5	0 - 0.1
Rabbit	0 - 0.2	0 - 0.1
Buffalo	0.1 - 0.5	0 - 0.1

It is recommended that each laboratory establish its own normal range representing its patient population.

PRESENTATION

REF	1126040035
Pack Size Σ	35 Tests
DL1 Direct Bilirubin Reagent	75 ml
DL2 Direct Nitrite Reagent	4 ml
TL1 Total Bilirubin Reagent	75 ml
TL2 Total Nitrite Reagent	4 ml
S Artificial Standard (10 mg/dl)	10 ml

COMPOSITION

Direct Bilirubin : Sulfanic Acid 17mmol; HCl 300 mmol.
Total Bilirubin : Sulfanic Acid 17mmol; HCl 300mmol; Caffeine 30mmol; Non Reactive Stabilizers and Preservatives.

STORAGE / STABILITY

Contents are stable at 23-29°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use. Do not pipette with mouth.

SAMPLE MATERIAL

Serum. Bilirubin is reported to be stable in the sample for 4 days at 2-8°C protected from light, as it is photosensitive.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Wavelength / filter : 546 nm / Yellow - Green
Temperature : R.T.
Light path : 1 cm

Direct Bilirubin Assay:
Pipette into clean dry test tubes labelled as Blank (B) and Test (T):

Addition Sequence	B (ml)	T (ml)
Direct Bilirubin Reagent (DL1)	1.0	1.0
Direct Nitrite Reagent (DL2)	--	0.05
Sample	0.1	0.1

Mix well and incubate at R.T. for exactly 5 mins. Measure the absorbance of the Test Samples (Abs.T) immediately against their respective Blanks.

Total Bilirubin Assay:
Pipette into clean dry test tubes labelled as Blank (B) and Test (T):

Addition Sequence	B (ml)	T (ml)
Total Bilirubin Reagent (TL1)	1.0	1.0
Total Nitrite Reagent (TL2)	--	0.05
Sample	0.1	0.1

Mix well and incubate at R.T. for 10 mins. Measure the absorbance of the Test Samples (Abs.T) immediately against their respective Blanks.

CALCULATIONS

Total or Direct Bilirubin in mg/dl = Abs. T x 13

QUALITY CONTROL

The following process is recommended for QC during the assay of Bilirubin. Define and establish acceptable range for your laboratory.
1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, re-calibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

LOD : 0.03 mg/dl
LOQ : 0.05 mg/dl
Lower Limit : 0.03 mg/dl
Higher Limit : 20 mg/dl

If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Interferences:
Sample when spiked with interferent such as upto 1000 mg/dl intralipid and 500 mg/dl haemoglobin does not affect the ability of the kit to determine the Bilirubin concentration.

Precision:
Within run
Direct

Within run	n	Mean	SD	% CV
Sample 1	10	1.17	0.03	2.37
Sample 2	10	1.76	0.02	1.23
Sample 3	10	2.20	0.05	2.25

Total

Within run	n	Mean	SD	% CV
Sample 1	10	1.75	0.03	1.55
Sample 2	10	5.75	0.03	0.58
Sample 3	10	6.15	0.03	0.47

Between run
Direct

Between run	n	Mean	SD	% CV
Sample 1	10	1.16	0.02	2.00
Sample 2	10	1.76	0.02	1.35
Sample 3	10	2.22	0.05	2.25

Total

Between run	n	Mean	SD	% CV
Sample 1	10	1.76	0.02	1.17
Sample 2	10	5.56	0.03	0.50
Sample 3	10	6.20	0.03	0.50

Method comparison:
Comparative studies were done to compare our reagent with another commercial Bilirubin Assay. No significant differences were observed. Details of the comparative studies are available on request.

NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. Only clean and dry glassware must be used. In case the exact wavelength is not available the artificial standard (S) may be used. Measure the absorbance of the artificial standard against distilled water with the appropriate filter and keep the same for future calculations by dividing the Abs.T with the Abs. of the Std. x 10. Discard the Artificial Standard after use.
In case of neonates where the sample quantity is a limitation, and the samples have high bilirubin (above 3 mg/dl), only 0.05 ml / 0.02 ml of the sample may be used for bilirubin estimation. The calculation factor in this case would be 24.9 / 60.5 respectively instead of 13. In case of using the standard the value of the same would be 19.1 / 46.5 mg/dl respectively instead of 10 mg/dl. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Jendrassik, L., Grof, P., (1938) Biochem. 2,297: 81.
2. Sherlock S. (1951) p.204 in Liver Disease, Churchill, London.
3. Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
4. Data on file: Coral Clinical Systems.

System Parameters

Reaction	: End Point	**Interval**	: ---
Wavelength	: 546 nm	**Sample Vol.**	: 0.10 ml
Zero Setting	: Sample Blank	**Reagent Vol.**	: 1.05 ml
Incub. Temp.	: R. T.	**Standard**	: ---
Incub. Time	: 5 min. / 10 min.	**Factor**	: 13
Delay Time	: ---	**React. Slope**	: Increasing
Read Time	: —	**Linearity**	: 20 mg/dl
No. of read.	: ---	**Units**	: mg/dl

SYMBOL KEYS

29°C / 23°C Store at 23-29°C	Manufacturer	This way up	**Modified Jendrassik & Grof's** Modified Jendrassik & Grof's Method
Use by (Last day of stated month)	Consult Instructions for use	DL1 Direct Bilirubin Reagent	TL1 Total Bilirubin Reagent
Date of Manufacture	REF Catalogue Number	DL2 Direct Nitrite Reagent	TL2 Total Nitrite Reagent
LOT Batch Number	IVD *In vitro* Diagnostic Medical Device	S Artificial Standard (10 mg/dl)	Σ Contains sufficient for <n> tests

Manufactured by:
Coral Clinical Systems
A Division of Tulip Diagnostics (P) Ltd.
BUILDING E, PLOT NO. M-46/47, PHASE III B, VERNA INDUSTRIAL ESTATE, VERNA, GOA-403 722, INDIA.
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