Total Bilirubin Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 342 µmol/L (20.0 mg/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Corrosion

INTENDED USE
For in vitro diagnostic use. For the quantitative determination of total bilirubin in serum.

SUMMARY AND EXPLANATION
Bilirubin, a product of red blood cell destruction, is a bile pigment normally found in the blood. The average life expectancy of red blood cells is 120 days. Approximately 6 gm of hemoglobin is released per day due to their disintegration. Reticuloendothelial cells from the spleen, liver, and bone marrow phagocytize aged red cells and convert the released hemoglobin to bilirubin.[1] Serum albumin links to bilirubin and transports it to the liver where it is metabolized.
Elevated serum bilirubin can indicate impairment of liver excretory function, excessive hemolysis, or biliary tract obstruction.[2] Hyperbilirubinemia can also be associated with obstructive jaundice, hemolytic and hepatic jaundice, infectious hepatitis, and pernicious anemia.

PRINCIPLE
This reagent is a variation of the classical method of Van den Bergh and Mueller.[3] Total bilirubin, both conjugated and free, is measured by using a stabilized diazonium salt of 3,5-dichloroaniline which reacts with bilirubin to form azobilirubin with maximum absorbance at 540 nm. Surfactants are used as reaction accelerators. The concentration of bilirubin present is directly proportional to the absorbance of the azobilirubin measured spectrophotometrically at 540 nm.

REAGENT COMPOSITION

Active Ingredient	Concentration
3,5-dichlorophenyldiazoniumtetrafluoroborate	0.36 mmol/L
surfactants	

Hazard Symbol: Corrosion
Signal Word: Danger

Hazard Statements
H318 Causes serious eye damage

Precautionary Statements - Prevention
Wear protective gloves/protective clothing/eye protection/face protection
Precautionary Statements - Response
Eyes
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 or doctor/physician
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
0.01% of the mixture consists of ingredient(s) of unknown toxicity
Other information
Causes mild skin irritation
Harmful to aquatic life
Refer to the product Safety Data Sheet for additional information.

REAGENT PREPARATION
Reagent is ready to use as supplied.

STORAGE AND STABILITY
The reagent is stable until the expiration date as stated on the label if stored at 2-8°C and kept tightly capped. Protect reagent from light.

Indications of Reagent Deterioration:
1. The reagent should be clear, colorless to pale yellow.
2. Turbidity or failure to achieve assigned values on assayed control sera could indicate deterioration.
3. If the reagent absorbance when determined manually is greater than 0.100 at 540 nm, the reagent may have deteriorated and should not be used.

SPECIMEN COLLECTION AND HANDLING
Serum: Fresh non-hemolyzed serum is the recommended sample.[1] Separate serum from cells promptly to minimize hemolysis.
Storage: Serum samples should be protected from light. Direct sunlight or white light exposure may cause a 50% decrease in bilirubin within one hour.[1] It is recommended that specimens be collected and processed as described in NCCLS[4] (H3, H4, H18) or equivalent publications. Serum bilirubin is stable up to one week if stored at 2-8°C and for approximately three months if stored frozen and protected from light exposure.[1]

MATERIALS PROVIDED
Total Bilirubin Reagent
MATERIALS REQUIRED BUT NOT PROVIDED
1. Thermo Data-Cal or equivalent.
2. Thermo Normal and Abnormal controls or equivalent.
3. Pipettes for accurately dispensing 1.0 mL volumes.
4. Micropipettes for dispensing 0.05 mL volumes.
5. Suitable manual instrument calibrated to read at 540 nm.
6. Hitachi 704® Analyzer, or equivalent, with manual and accessories.

ASSAY PROCEDURE

HITACHI 704 INSTRUMENT PARAMETERS

Test		TBil
Assay		1 POINT:6-0
Sample Volume		20
R1 Volume		400-20-NO
R2 Volume		0-20-NO
Wavelength	660/546	
Calib. Method		Linear-0
STD (1) Conc.-Pos.		0-1
STD (2) Conc.-Pos.		( )-2
STD (3) Conc.-Pos.		( )-3
STD (4) Conc.-Pos.		( )-4
STD (5) Conc.-Pos.		( )-5
STD (6) Conc.-Pos.		( )-6
Unit		mg/dL
SD Limit		0.1
Duplicate Limit		200
Sensitivity Limit		0
ABS Limit (Inc./Dec)		0 (Inc)
Prozone Limit		0 (Lower)
Expected Value		0.0 - 1.5
Instrument Factor		1.00

The above parameters should be used when programming the Hitachi 704. Consult your instrument manual for further instructions.

MANUAL PROCEDURE
1. For each sample, dispense 1.0 mL of Total Bilirubin Reagent into labelled test tubes.
2. Add 0.05 mL of calibrator, control, and sample to its respective tube. Mix immediately. Use 0.05 mL of deionized water as sample for reagent blank.
3. Incubate at reaction temperature for 5 minutes.
4. Set the wavelength of the instrument at 540 nm. Zero with reagent blank.
5. Read and record absorbance of samples.

SAMPLE BLANK
Sample blanks are required for some turbid, icteric, and hemolyzed samples. This includes many control sera and calibrators.
1. For each sample to be blanked, dispense 1.0 mL normal saline into labelled test tubes.
2. Add 0.05 mL of each sample to be blanked. Mix and incubate for 5 minutes at the reaction temperature.
3. Set the wavelength of the instrument at 540 nm. Zero with normal saline.

Thermo SCIENTIFIC

4. Read and record absorbance of blank. Subtract this absorbance from Step 5 in the Manual Procedure Section. The corrected absorbance is used in the calculation of results.

STABILITY OF FINAL REACTION MIXTURE
The final colored product is stable for 60 minutes at controlled room temperature (15-30°C). The Hitachi 704 reads each standard and sample at the same time.

CALCULATION OF RESULTS
The Hitachi 704 analyzer automatically calculates the results.
Use the equation below to calculate the total bilirubin concentration of a sample when using the manual procedure.

$$\text{Total Bilirubin} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of Unknown	=	0.052
Absorbance of Calibrator	=	0.180
Calibrator Value	=	5.2 mg/dL

$$\text{Total Bilirubin} = \frac{0.052}{0.180} \times 5.2 = 1.5 \text{ mg/dL}$$

NOTE
Unit conversion: mg/dL x 17.1 = µmol/L.

CALIBRATION
It is not necessary to determine a standard curve with this procedure, since the reaction is linear to 342 µmol/L (20.0 mg/dL). However, a reagent blank and calibrator should be employed with each set of unknowns assayed.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above the upper limit or below the lower limit of the established ranges indicate the assay may be out of control.
The following corrective actions are recommended in such situations:-
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or the local distributor.

LIMITATIONS
1. See Storage and Stability, Deterioration, Specimen Collection, Interfering Substances, Sample Storage, Stability of Final Reaction Mixture and Linearity sections for limitations to this procedure.
2. Bilirubin calibrators in chloroform may not be used in this procedure since chloroform miscible solvents are not employed.
3. Bilirubin is extremely light sensitive. Calibrator, control and unknown specimens must be stored protected from light sources for optimal stability.

INTERFERING SUBSTANCES
1. Lipemia does not interfere with this procedure when used on the Hitachi 704 or with a manual instrument up to 1335 mg/dL of triglyceride.
2. When used with the Hitachi 704, hemoglobin levels of 83 mg/dL or above cause significant interference at a bilirubin level of 1.1 mg/dL. A hemoglobin level of up to 398 mg/dL does not significantly interfere at a bilirubin level of 5.1 mg/dL.
   When used manually, hemoglobin levels of 83 mg/dL or above cause significant interference at a bilirubin level of 1.3 mg/dL. A hemoglobin level up to 204 mg/dL does not significantly interfere at a bilirubin level of 5.3 mg/dL.
3. Young has reviewed drug effects on serum bilirubin levels.[5]

EXPECTED VALUES
NORMAL RANGE[6] 0.0 to 1.5 mg/dL

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population it serves.[7]

PERFORMANCE DATA
The Performance Characteristics were established on a Hitachi 704 analyzer and using the manual procedure. The user should establish performance characteristics if the product is used on another analyzer.

PRECISION
Within-run reproducibility was determined by assaying three levels of control sera 20 times.

Within-Run/Hitachi 704	MEAN	STD. DEV.	CV%
LOW	0.60 mg/dL	0.00	0.00
MODERATE	5.90 mg/dL	0.05	0.85
HIGH	15.20 mg/dL	0.10	0.66

Within-Run/Manual	MEAN	STD. DEV.	CV%
LOW	1.0 mg/dL	0.05	5.00
MODERATE	5.9 mg/dL	0.13	2.20
HIGH	14.7 mg/dL	0.23	1.56

Run-to-run reproducibility was obtained by assaying three levels of control sera as single points for 10 runs, over a 4 day period.

Run-To-Run/Hitachi 704	MEAN	STD. DEV.	CV%
LOW	0.63 mg/dL	0.07	1.11
MODERATE	5.70 mg/dL	0.07	1.20
HIGH	14.50 mg/dL	0.17	1.20

Run-To-Run/Manual	MEAN	STD. DEV.	CV%
LOW	1.1 mg/dL	0.07	6.40
MODERATE	6.0 mg/dL	0.05	0.83
HIGH	15.2 mg/dL	0.32	2.10

COMPARISON
A comparison of the Thermo Total Bilirubin #1245 (y) with Total Bilirubin #1240 (x) was performed on the Hitachi 704 on 169 samples in a range of 0.1 to 19.5 mg/dL. The resultant correlation equation is y = 1.00x - 0.04 , with a correlation coefficient of 0.999. A comparison of the Thermo Total Bilirubin #1245 (y) with Total Bilirubin #1240 (x) was performed manually on 167 samples in a range of 0.2 mg/dL to 21.6 mg/dL. The resultant correlation equation is y = 0.99x - 0.01, with a correlation coefficient of 0.999.

LINEARITY
Linearity extends to 342 µmol/L (20.0 mg/dL). Samples exceeding linearity should be diluted with normal saline and repeated. Multiply the concentration by the dilution factor when calculating the unknown.

SENSITIVITY
Based on the Hitachi 704 instrument resolution of A = 0.001, the Thermo Total Bilirubin Reagent has a sensitivity of 0.07 mg/dL. The analytical sensitivity is 0.1 mg/dL.
Based on a manual instrument resolution of A = 0.001, the Thermo Total Bilirubin Reagent has a sensitivity of 0.03 mg/dL. The analytical sensitivity is 0.1 mg/dL.

BIBLIOGRAPHY

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7. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

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JL840886-en (R0)

REF

Reorder Information

Catalogue No.	Configuration
1245-250	2 x 125 mL