TECO DIAGNOSTICS
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BILIRUBIN TOTAL
TC MATRIX

INTENDED USE
For the quantitative determination of total bilirubin concentration in serum or plasma on TC Matrix analyzers.

SUMMARY AND EXPLANATION OF THE TEST
Bilirubin, in bile, is derived primarily from the breakdown of hemoglobin when senescent red blood cells are phagocytized. Normally, about 6 to 6.5 g of hemoglobin in aged red blood cells are broken down daily in an adult to form about 220 mg of bilirubin; another 50 to 60 mg of bilirubin originates from other sources. The exact mechanism to form bilirubin is not well understood. But it is known that the heme group of hemoglobin converts to bilirubin in reticuloendothelial system, which binds to albumin in plasma and esterified to bilirubin diglucuromide (BDG), which is then secreted from liver as a waste product. It is present in serum in the free and conjugated forms. An increase in the formation or retention of bilirubin in the body results in increased levels of serum Bilirubin and jaundice. This hyperbilirubinemia is classified as either pre-hepatic, hepatic or post-hepatic depending on the principal cause of condition. Therefore, determination of the total bilirubin and its conjugated (direct) bilirubin is important for the differential diagnosis of hyperbilirubinemia.

Sulfanilic acid reacts with sodium nitrite to give diazotized sulfanilic acid. In the presence of dimethyl sulfoxide, both direct and indirect bilirubin couples with diazotized sulfanilic acid to give a colored complex azobilirubin which is measured spectrophotometrically at 520 nm.

The TC Matrix System automatically proportions the appropriate sample and reagent volumes into the cuvette. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of total bilirubin in the sample and is used by the TC Matrix System to calculate and express total bilirubin concentration.

Total Bilirubin + diazo + H^+ ——→ azobilirubin (Blue color)

REAGENT CONTENTS:
Each kit contains: Four Total Bilirubin Reagent 1 (4×30 ml)
Four Total Bilirubin Reagent 2 (4×5 ml)
Instruction Insert

REAGENT PREPARATION
No preparation is required.

REAGENT COMPOSITION
Sulfanilic acid: 27 mM
Sodium nitrite: 0.12 mM
HCl: 51 mM
DMSO: 45% (v/v)
Also non-reactive chemicals for optimal system performance.

REAGENT STORAGE AND STABILITY
Total Bilirubin Reagent stored unopened at 2-8 °C is stable until the expiration date shown on the bottle label. Once opened, Total Bilirubin Reagent is stable for 30 days, or until the expiration date shown on the label, whichever occurs first. DO NOT FREEZE.

SPECIMEN COLLECTION AND HANDLING
1. The test can be performed on serum or plasma. For serum, blood is drawn into a tube which does not contain anticoagulant and it is allowed to clot. The serum should then be separated from the clot within two hours from the time of collection.
2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum and plasma should be stored at 2ºC to 8ºC. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15ºC to -20ºC. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
3. For plasma, add whole blood directly into a tube containing anticoagulant. Acceptable anticoagulants are listed in the "LIMITATIONS" section.

CALIBRATION
1. Calibrator required: TECO MULTI Calibrator (not provided in kit)
2. The system must have a valid calibration in memory before controls or patient samples can be run.
3. The TC Matrix system will automatically perform checks on the calibration and produce data at the end of calibration.

Note: Refer to the TC Matrix manual for further instructions on calibrating the instrument.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

TECO MULTI Calibrator
At least two levels of control material.

LIMITATIONS
The anticoagulants Potassium Oxalate, Sodium Fluoride, EDTA, Lithium Heparin, Sodium Heparin, Ammonium Heparin and Sodium Citrate were found to be compatible with this method.

INTERFERENCE
1. Hemoglobin may interfere with this methodology.
2. Lipemic samples >3+ should be ultra-centrifuged and the analysis performed on the infranate.
3. On this method, refer to the work of Young for a review of drug and comprehensive list of substances which affect total bilirubin level.

EXPECTED VALUE
0.2 to 1.0 mg/dL or 3.4 to 17.1µmol/L

PRECAUTIONS:
1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH Biosafety in Microbiological and Biomedical Laboratories manual, and in accordance with national or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control tests to ensure the results are reliable before testing the specimens.

PROCEDURES

TEST NAME:	TBIL	R1:	300
TEST NO.		R2:	30
FULL NAME:	Total Bilirubin	SAMPLE VOLUME:	10
REFERENCE NO.:		R1 BLANK:	/
ANALY.TYPE:	Endpoint	MIX REAG. BLANK:	0.1 - 1.5
PRI. WAVE :	546 nm	CONCENTRATION:	/
SECON. WAVE:		LINEARITY LIMIT:	0.1 - 30
TREND:	Increase	SUBSTRATE LIMIT:	/
REACT. TIME:	0 - 18	FACTOR:	/
INCUBATE TIME:	4	PROZONE CHECK:	/
UNIT:	mg/dl	Q1: / Q2: / Q3: / Q4: /	
PRECISION:	0.1	PC: / ABS.: /	
Calibration Type: Calibrate+ Reag.Blank		Calibration Rule: Two-point linear	

PERFORMANCE CHARACTERISTICS

Analytical Range: 0.1-30 mg/dl

For Total Bilirubin analysis by Total Bilirubin Reagent on TC Matrix System, this method has been demonstrated to be linear from 0.1- 30 mg/dl.

Accuracy: Comparison study was performed on TC Matrix System for 40 samples. Beckman Coulter Total Bilirubin Reagent was used to compare with Total Bilirubin Reagent. The results of this study yielded a correlation coefficient of 0.98 with a regression equation of y = 0.98X -0.4

Precision: Within Run precision for Total Bilirubin Reagent Set was determined following a modification of NCCLS EP5-A. Two commercial human serum samples, Total Bilirubin Calibrators, were assayed on TC Matrix System for 25 times.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	1.1	6.0
Standard Deviation (mg/dl)	0.07	0.3
Coefficient of Variation (%)	4.7	4.8

Run-to-Run precision for Total Bilirubin Reagent was determined following a modification of NCCLS EP5-A. Two commercial human serum samples, Total Bilirubin Calibrators, were assayed on TC Matrix Systems five times per day for five days for the total of 25 values.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	1.1	6.0
Standard Deviation (mg/dl)	0.08	0.3
Coefficient of Variation (%)	5.1	4.9

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Manufactured by:

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