TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

BILIRUBIN DIRECT
TC MATRIX-160

INTENDED USE
For the quantitative determination of direct (conjugated) bilirubin concentration in serum or plasma on TC Matrix analyzer.

SUMMARY AND EXPLANATION OF THE TEST
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. Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560 nm in the aqueous solution. The intensity of the color produced is directly proportional to the amount of direct bilirubin concentration present in the sample.
The TC Matrix System automatically proportions the appropriate sample and reagent volumes into the cuvette. The system monitors the change in absorbance at 560 nanometers. This change in absorbance is directly proportional to the concentration of direct bilirubin in the sample and is used by the TC Matrix System to calculate and express direct bilirubin concentration.

Direct Bilirubin + diazo + H^+ $\longrightarrow$ azobilirubin (red color)

REAGENT PREPARATION
No preparation is required.

REAGENT COMPOSITION
Sulfanilic acid: 27 mM
Sodium nitrite: 0.12mM
HCl: 51 mM
Also includes non-reactive chemicals for optimal system performance

REAGENT STORAGE AND STABILITY
Direct Bilirubin Reagent stored unopened at 15ºC to 30ºC is stable until the expiration date shown on the bottle label. Once opened, Teco Direct Bilirubin Reagent is stable for 30 days at 15ºC to 30ºC, or until the expiration date shown on the bottle label, whichever date occurs first.
DO NOT FREEZE.

SPECIMEN COLLECTION AND HANDLING
1. The test can be performed on serum or plasma. For serum, whole blood is drawn into a tube which does not contain anticoagulant and is allowed to clot. For plasma, whole blood is collected into a tube containing anticoagulant. Acceptable anticoagulants are listed in the "LIMITATIONS" section. It is recommended that the serum or plasma be physically separated from contact with cells 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 can not be completed within 8 hours, serum and plasma should be stored at 2ºC to 8ºC. If assays can not be 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. Bilirubin is photosensitive. Protect samples from light.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT
TECO MULTI Calibrator
At least two levels of control material.

CALIBRATION
1. The system must have a valid calibration in memory before controls or patient samples can be run.
2. 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.

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.

INTERFERENCES
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 drugs and comprehensive list of substances which effect Direct Bilirubin level.

EXPECTED VALUES
0.0 to 0.2 mg/dL or 0.0 to 3.4µmol/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 manual *Biosafety in Microbiological and Biomedical Laboratories*, and in accordance with national or local regulations related to the safety precautions of such materials.
3. As part of quality control, with each new calibration and prior to testing specimen samples, each laboratory should analyze at least two levels (normal and abnormal) of control material having known direct bilirubin concentrations.

PROCEDURE

Test Name:	D-BIL	R1:	147
Full Name:	Bilirubin Direct	R2:	32
Pri. Wave:	546 nm	SAMPLE VOLUME:	5.7
Sec. Wave:	700 nm	Calibration Type:	2 point linear
Assay/ Point:	2-point end	K Value:	/
Start - End:	10 - 22	Point:	2
Decimal place:	2	Blank Type:	Reagent
Unit:	mg/dL	Point 0 (Blank) Con.:	0.0
Linearity Range:	0.1000 - 20.000	Point 1 (STD) Con.:	Calibrator / standard
Correlation Factor:	1.0000 - 0.0000		

PERFORMANCE CHARACTERISTICS

Analytical Range: 0.1-20 mg/dL

For Direct Bilirubin analyte using Direct Bilirubin Reagent on TC Matrix System, this method has been demonstrated to be linear from 0.1- 20 mg/dl.

Accuracy: Comparison study was performed on TC Matrix System from 40 samples. Beckman Coulter Direct Bilirubin Reagent was used to compare with Teco Diagnostics' Direct Bilirubin Reagent. The results of this study in yield a correlation coefficient of 0.99 with a regression equation of y=0.99x -0.01

Precision: Within Run precision for Direct Bilirubin Reagent was determined following a modification of NCCLS EP5-A. Two commercial human sera, Direct Bilirubin Calibrator, were assayed on TC Matrix System for 25 times.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	0.3	1.4
Standard Deviation (mg/dl)	0.01	0.02
Coefficient of Variation (%)	4.5	4.0

Run-to-Run precision for Direct Bilirubin Reagent was determined following a modification of NCCLS EP5-A. Two commercial human sera, Direct Bilirubin Calibrator, were assayed on TC Matrix System five times per day for five days for the total of 25 values.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	0.3	1.4
Standard Deviation (mg/dl)	0.01	0.02
Coefficient of Variation (%)	4.5	4.0

REFERENCES:

1. Malloy, H.T. and Evelyn, K.A. 1960, j.Biol. Chem. 119, 48.
2. Valdez, O.S.et.al.1971 J.Pediat 79, 1015.
3. Tietz, N.W., "Specimen Collection and Processing; Sources of Biological Variation," Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders, Philadelphia, PA (1994).
4. National Committee for Clinical Laboratory Standards. Approved Guideline, NCCLS publication C28-A, Villanova, PA (1994).
5. Henry, J. B., ed., Clinical Diagnostics and Management by Laboratory Methods, 18th Edition, W.B. Saunders, Philadelphia.
6. Tietz, N.W., ed., Clinical Guide to Laboratory Tests, 2nd Edition, W.B. Saunders, Philadelphia, PA (1990)
7. National Committee for Clinical Laboratory Standards, Method Comparison and Bias Estimation Using Patient Samples; Tentative Guideline, NCCLS Publication EP9-T, Villanova, PA (1993).
8. National Committee for Clinical Laboratory Standards, Precision Performance of Clinical Chemistry Devices; Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).
9. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol. 4, No, June 1984.
10. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd. Ed., AACC Press, Washington DC, 1990, 3:104-106.
11. Young, D. S., Effects of Preanalytical Variables on Clinical Laboratory Tests, 2nd Edition, AACC Press, Washington, D. C. (1997).

B538-TC1: 10/2023

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.