TECO DIAGNOSTICS
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BILIRUBIN DIRECT
TC MATRIX-240/480

INTENDED USE
The test is applied for the quantitative determination of direct bilirubin in serum and plasma.

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 2,4-dichloroaniline 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.

TEST PRINCIPLE (*Colorimetric diazo method)*
Direct bilirubin in the sample to be measured reacts with diazotized 2,4-dichloroaniline in the reagent to form azobilirubin, a diazo molecule with an intense red color in acidic medium. This color is measured photometrically by absorbance reading at a wavelength of 546 nm (520-560 nm) and is directly proportional to the concentration of direct bilirubin in the sample.

Direct Bilirubin + diazo + H^+ ⟶ azobilirubin (red color)

REAGENT COMPONENTS
Reagent 1

Sodium chloride	: ≤ 0.01 M
EDTA	: ≤ 0.30 M

Reagent 2

Diazotized 2,4-dichloroaniline	: ≤ 0.12 M
Hydrochloric acid	: ≤ 0.22 M
EDTA	: ≤ 0.01 M

Reagent preparation
No preparation is required.

REAGENT STORAGE AND STABILITY
Reagents are stable at 2-8˚C until the expiration date stated on the label which is only for closed vials.
Once opened vials are stable for 30 days at 2-8°C in optimum conditions.
On board stability is strongly related to auto analyzers' cooling specification and carry-over values.
Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.

Direct bilirubin activity stability in serum and plasma: 2 days at 20-25 ºC, 7 days at 2-8ºC and 3 months at -20°C

SPECIMEN COLLECTION AND HANDLING
Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin, Na heparin, K2-EDTA or K3-EDTA must be preferred. Contact with light must be avoided. Non-lipemic samples must be used. Multiple sample freezing and thawing should be avoided

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT
Arcal Auto Calibrator
At least two levels of control material.
Note: Refer to the TC Matrix manual for further instructions on calibrating the instrument.

Unit Conversion:
mg/dL x 17.1 = µmol/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator. Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used.
Arcon N Level 1 Control- Lyophilized
Arcon P Level 2 Control- Lyophilized

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS
Adults : ≤ 0.40 mg/dL

PROCEDURE
Settings for TC-Matrix 240/480

Test Name:	D-BIL	R1:	147
Full Name:	Bilirubin Direct	R2:	32
Pri. Wave:	576 nm	SAMPLE VOLUME:	5.7
Sec. Wave:	700 nm	Calibration Type:	2 point linear
Assay/ Point:	2-point end	K Value:	/
Start - End:	9 - 22	Point:	2
Decimal place:	2	Blank Type:	Reagent
Unit:	mg/dL	Point 0 (Blank) Con.:	0.0
Linearity Range:	0.1000 - 13.000	Point 1 (STD) Con.:	Calibrator / standard
Correlation Factor:	1.0000 - 0.0000		

PERFORMANCE CHARACTERISTICS
Measuring Interval
According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).
The determined analytic measuring interval for Bilirubin Direct is 0.09 – 13 mg/dL.

Detection Capability
Limit of Detection (LoD): 0.04 mg/dL
Limit of Quantitation (LoQ): 0.09 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.

Linearity

This method shows measurement linearity in the activities up to 13 mg/dL. Autoanalyzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within- Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.

Table 1. Direct Bilirubin Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.44 mg/dL	0.01	1.84	80
4.47 mg/dL	0.02	0.49	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.

Table 2. Direct Bilirubin Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.44 mg/dL	0.01	2.40	80
4.47 mg/dL	0.16	3.60	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.

Method Comparison

As a result of the statistical evaluation of the method comparison data:
Passing-Bablok equation: y= 0.92x + 0.05 mg/dL
r= 0.998

Interference

Endogenous interferant and analyte concentrations that have been used in the Direct Bilirubin scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Direct Bilirubin interference scanning test is appropriate, is determined as ±10%.

In Direct Bilirubin test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant Concentration	Direct Bilirubin Target (mg/dL)	N*	Observed Recovery %
Hemoglobin 180 mg/dL	0.23	3	100

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[27]

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only. Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

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