MEDICA

IVD CE

REF 10207-4 4 x 29 mL / 8 mL

TOTAL BILIRUBIN (TBIL)

Wedges each contain usable volumes of 29 mL of R1 reagent and 8 mL of R2 reagent.

INTENDED USE

The EasyRA TIBL reagent is intended for the quantitative measurement of total bilirubin in the human serum or plasma of adults, using the MEDICA "EasyRA® Clinical Chemistry Analyzer". Bilirubin measurements are used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder blockages.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Bilirubin is a pigment formed from hemoglobin, and present in the serum as the result of red cell destruction. The increased level of bilirubin may be a result of hemolytic processes, liver disease or biliary tract disorders. Bilirubin exists in two forms; unconjugated (indirect) and conjugated (direct). Unconjugated bilirubin is transported to the liver, where it is bound by albumin and becomes conjugated (direct) with glucuronic acid, which is eventually excreted. Unconjugated bilirubin is not soluble in aqueous solution and requires alcohols and other solvents for solubilization. Assays involving these types of solvents provide the total bilirubin amount. Bilirubin mono and diglucuronide conjugates are water-soluble and assays of these types measure direct bilirubin. The traditional method involves the reaction of bilirubin with a diazo reagent, (e.g. 2,4-dichlorophenyldiazonium salt) to form the colored compound azo-bilirubin. Various additives such as ethanol [1], caffeine [2] and DMSO [3] were added to accelerate the reaction and formation of the azo-bilirubin compound. Also included was the addition of surfactants as solubilizing agents [4].

PRINCIPLE OF THE PROCEDURE

This endpoint reaction method measures total bilirubin (conjugated and unconjugated) binding to the 2,4-dichlorophenyldiazonium salt in the presence of surfactant to form azo-bilirubin.

$$\text{Total bilirubin} + \text{2,4-dichlorophenyldiazonium} \xrightarrow{\text{Surfactant}} \text{Azo-bilirubin}$$

The increase in absorbance at 550 nm is directly proportional to the Total Bilirubin concentration in the sample.

REAGENTS

TBIL Reagent (R1):

NaCl	154 mmol/L
HCL	190 mmol/L
Surfactants and preservatives	

TBIL Reagent (R2):

HCL	417 mmol/L
2,4-dichlorophenyldiazonium salt	5 mmol/L

Surfactant

Precautions

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (*CLSI,* GP17-A2).
1. The reagents (R1 and R2) are acidic solutions. Do NOT inhale or swallow, and avoid any contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Any contact with the skin, should be washed immediately with water for 10 minutes. If swallowed, seek medical advice immediately. Refer to the Safety Data Sheet for risk, hazard and safety information.
2. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
3. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2° – 8°C. The reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY
Clear unhemolyzed serum or plasma should be used. Protect samples from direct sun and artificial light as direct (unconjugated) bilirubin is unstable [5]. This assay should be done within 2 hours from collection, as bilirubin is unstable in the sample. If the samples cannot be assayed within this time, store samples for 3 days at 2 – 8°C[6]. Samples can also be stored for 3 months at –70ºC. Lithium heparin coated tubes may be used for plasma collection.

PROCEDURE

Materials Provided
Medica TBIL Reagent Wedge, REF 10207

Additional materials required

Medica EasyCal Chemistry, REF 10651
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the refrigerated reagent area. Opened reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge (28 days maximum) or when removed and stored refrigerated at 2°-8°C (capped) after first opened.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration
Medica EasyCal Chemistry (REF 10651) is recommended for the calibration of the assay. The calibration interval (7 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control
It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the Medica EasyRA Chemistry Analyzer calculates the Total Bilirubin concentration from the ratio of the corrected unknown sample's absorbance to the corrected absorbance of the calibrator multiplied by the calibrator value.

$$\text{TBIL (mg/dL)} = \frac{[(A_{U_{550}} - A_{U_{600}}) - (A_{RBlk_{550}} - A_{RBlk_{600}})] - [(A_{U_{550}} - A_{U_{600}})_{SBlk} - (A_{RBlk_{550}} - A_{RBlk_{600}})_{SBlk}] \times dF}{[(A_{C_{550}} - A_{C_{600}}) - (A_{RBlk_{550}} - A_{RBlk_{600}})] - [(A_{C_{550}} - A_{C_{600}})_{SBlk} - (A_{RBlk_{550}} - A_{RBlk_{600}})_{SBlk}] \times dF} \times \text{CalValue}$$

Where A_U and A_C are the absorbance values of the unknown and the calibrator, respectively; A_{RBlk} is absorbance of the reagent blank; S_{Blk} is sample blank; and "Cal Value" is the concentration of Total Bilirubin in the calibrator (mg/dL). Since the volume of the reaction is changed with the delayed addition of the R2 reagent, there is a dilution correction factor (dF) included in the calculation.

The sample blank is corrected for the dilution factor difference introduced by using R1 vs. (R1 and R2) using:

$$df = \frac{\text{Reag}_1 \text{ Vol} + \text{Dil. Vol.} + \text{Samp. Vol.}}{\text{Total Volume}}$$

Expected Values [10]
The reference range for TBIL in serum is as follows:

Adult: 0.2 – 1.0 mg/dL

These values are guidelines. It is recommended that each laboratory establish its own range of normal values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
Only unhemolyzed serum or plasma samples should be used. This assay has not been evaluated in neonates.

The EasyRA Analyzer flags any result above 20 mg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will effectively extend the reportable range of the TBIL test to 40 mg/dL

PERFORMANCE CHARACTERISTICS [9]

Reportable Range
The reportable range is 0.08 to 20 mg/dL. Extended range is 0.08 to 40 mg/dL when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for TBIL (y) on the EasyRA Analyzer to the performance of a similar TBIL reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the Roche COBAS MIRA Analyzer.

Number of Samples	65	Range of Samples	0.05 to 19.8 mg/dL
Slope	1.0043	y Intercept	-0.1966
Correlation Coefficient	0.9957	Regression Equation:	Y = 1.0043*X – 0.1966

** Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.*

The following table lists the data obtained in a comparison of matched serum (x) and plasma (y) samples using the Medica Reagent for TBIL on the EasyRA Analyzer. The data below represents a single plasma determination vs. The average of two replicate serum samples.

Number of Samples	62	Range of Samples	0.17 to 16.78 mg/dL
Slope	0.9952	y Intercept	-0.0079
Correlation Coefficient	0.9998	Regression Equation	Y = 0.9952*X – 0.0079

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
3.36	0.02	0.67
1.66	0.02	1.13
0.44	0.01	2.64

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
3.36	0.05	1.35
1.66	0.03	2.09
0.44	0.02	3.81

Linearity (CLSI, EP6-A) [9]
Linear from 0.08 to 20 mg/dL based on the linear regression Y = 0.98*X + 0.0921.

Limit of Blank (LOB):	0.04 mg/dL	(CLSI, EP17-A)
Limit of Detection (LOD):	0.06 mg/dL	(CLSI, EP17-A)
Limit of Quantitation (LoQ):	0.05 mg/dL	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as "no significant interference".
There is significant interference at hemoglobin levels above 125 mg/dL.
There may be significant interference from triglycerides. Do not use Lipimic samples.

There is significant positive interference from indocyanine green.
Samples containing elevated levels of Immunoglobulin M (IgM) or samples from patients with Waldenstrom's Macroglobulinemia may produce unreliable results.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests[7].

References

1. Malloy H.T. and Evelyn, K.A., *The Determination of Bilirubin with the Photoelectros Colorimeter*., J.Biol. Chem 119:481-490 (1973).
2. Jendrassik, L and Grof.P. *Vereinfachte, Photometrische Methoden zur Bestimmung des Blubilirubins*, Bichem. A. 297: 81-89 (1938)
3. Walters, M. and Gerarde, H. *An Ultramicromethod for the Determination of Conjugated and Total Bilirubin in Serum or Plasma*. Microchem. J. 15:231-243 (1970).
4. Winsten, J. and Cehelyk, B., *A Rapid Micro Diazo Technique for Measuring Total Bilirubin*., Clin. Chem. Acta 25: 441-446 (1969).
5. Henry, RJ. *Clinical Chemistry, Principles and Technics,* Haggerstown, MD: Harper and Row, Publishers 1974:1058.
6. Tietz NW. *Textbook of Clinical Chemistry*, WB Saunders and Co., Philadelphia, PA, 1986: p1388.
7. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
8. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.
9. Data on file at Medica
10. Burtis, C.A. and Ashwood, E.R. (Eds),Tietz Textbook or Clinical Chemistry, 2nd edition, W.B. Saunders Co., Philadelphia (1994
11. NCCLS EP9-P
12. National Committee for Clinical Laboratory Standards, *User Evaluation of Precision Performance of Clinical Chemistry Devices* (NCCLS) Document EP5-T2 (ISBN 1-56238-145-8), 1992.

EasyRA Assay Parameters (TBIL)

Primary Wavelength (nm)	550
Secondary Wavelength (nm)	600
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Yes (with each calibration)
Sample Blank	Yes
Reaction Time	5.6 min
Calibration interval (maximum)	7 days
Reagent on-board stability	28 days

Serum/Plasma

Sample volume (µl)	8.0
Diluent 1 volume (µl)	15
Diluent 2 volume (µl)	15
Reagent volume R1 (µl)	150
Reagent volume R2 (µl)	38
Decimal Places (default values)	2
Units (default values)	mg/dL
Dilution	1:1 (to extend measuring range)
Linearity	0.08 to 20 mg/dL

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