

REF 10212-4 4 x 29 mL / 8 mL

DIRECT BILIRUBIN (DBIL)

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

INTENDED USE

The EasyRA DBIL reagent is intended for the quantitative determination of direct bilirubin in the human serum and 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 standard method described by Ehrlich ¹ involves the reaction of bilirubin with a diazo reagent (e.g. diazotized sulfanilic acid) to form the colored compound azo-bilirubin. This assay measures direct (conjugated) bilirubin based on its combination with a diazonium salt in the presence of sulfamic acid to form azo-bilirubin.

PRINCIPLE OF THE PROCEDURE

This endpoint reaction is based on using a 2,4-dichlorophenyldiazonium salt as the diazo reagent:

Direct bilirubin + 2,4-dichlorophenyldiazonium → Azobilirubin salt

Direct bilirubin (conjugated) binds with the diazo reagent to form azobilirubin. The increase in absorbance measured at 550 nm is proportional to the direct bilirubin concentration.

REAGENTS

DBIL Reagent (R1):

EDTA-Na ₂	0.1 mmol/L
NaCl	154 mmol/L
Sulfamic acid	100 mmol/L

DBIL Reagent (R2):

2,4-dichlorophenyldiazonium salt	5 mmol/L
HCL	0.9 mol/L
EDTA-Na ₂	0.13 mmol/L

Precautions

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. 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.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. 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 ². 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 up to 3 days at 2 – 8°C³. Samples can also be stored for 3 months at –70°C. Lithium heparin coated tubes may be used for plasma collection.

PROCEDURE

Materials Provided

Medica DBIL Reagent Wedge, REF 10212

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 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 (30 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 EasyRA 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 (30 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 EasyRA Analyzer calculates the direct bilirubin concentration from the ratio of the corrected absorbance (subtracting the absorbance of the reagent blank and sample blank) of the sample to the similarly corrected absorbance of the calibrator (after subtracting the absorbance of the reagent blank and sample blank) multiplied by the concentration of the calibrator.

$$\text{DBIL (mg/dL)} = \frac{[(A_{U_{550}} - A_{U_{700}}) - (A_{R_{Blk}_{550}} - A_{R_{Blk}_{700}})] - [(A_{U_{550}} - A_{U_{700}})_{SB_{Ik}} - (A_{R_{Blk}_{550}} - A_{R_{Blk}_{700}})_{SB_{Ik}}] \times dF}{[(A_{C_{550}} - A_{C_{700}}) - (A_{R_{Blk}_{550}} - A_{R_{Blk}_{700}})] - [(A_{C_{550}} - A_{C_{700}})_{SB_{Ik}} - (A_{R_{Blk}_{550}} - A_{R_{Blk}_{700}})_{SB_{Ik}}] \times dF} \times \text{CalValue}$$

Where A_U is the absorbance of the unknown, $A_{R_{Blk}}$ is the absorbance of the reagent blank associated with the unknown sample, SB_{Ik} is the absorbance of the sample blank associated with the unknown sample. All absorbances with a subscript "C" are associated with the calibrator. As a consequence of the delayed addition of the R2 reagent, there is a dilution correction factor (dF) included in the calculation.

Expected Values ³

The reference range for DBIL in serum is as follows:

Adult: 0.0 – 0.20 mg/dL (0.0 – 3.4 µmol/L)

These values are guidelines. It is recommended that each laboratory establish its own range of expected 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 10 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 extend the reportable range of the DBIL test to 20 mg/dL.

PERFORMANCE CHARACTERISTICS ⁴

Reportable Range

The reportable range is 0.06 to 10 mg/dL. Extended range is 0.06 to 20 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 DBIL (y) on the EasyRA Analyzer utilizing a primary wavelength of 550 nm and a secondary wavelength at 700 nm to the performance of the same DBIL reagent (x) on the EasyRA Analyzer utilizing a primary wavelength of 550 nm only. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the EasyRA Analyzer.

Number of samples	53	Range of Samples	0.06 to 9.50 mg/dL
Slope	1.0376	y Intercept	-0.0456
Correlation Coefficient	0.9986	Regression Equation	$Y = 1.0376 * X - 0.0456$

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

Number of samples	52	Range of Samples	0.07 to 9.95 mg/dL
Slope	1.0064	y Intercept	0.01
Correlation Coefficient	0.9992	Regression Equation	$Y = 1.0064 * X - 0.01$

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 %
2.47	0.04	1.59
1.31	0.02	1.83
0.20	0.01	5.49

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
2.47	0.05	2.09
1.31	0.02	1.87
0.20	0.01	6.93

Linearity (CLSI, EP6-A)

Linear from 0.06 to 10 mg/dL, based on the linear regression $Y = 0.98 * X + 0.0365$.

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 negative interference from hemoglobin. **Only** unhemolyzed serum or plasma samples should be used.

For DBIL of 2.55 mg/dL, no significant interference was found in levels up to 460 mg/dL of triglycerides (using Intralipid*). Medica recommends that visibly turbid samples not be used. 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.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests ^{5,6}.

REFERENCES

1. Ehrlich, P., *Sulfadiazobenzol, ein Reagens auf Bilirubin*. *Centr. Klin. Med.* 4; 721-723 (1883).
2. Gordon, E.R., *The Conjugate of Bilirubin*. *Hepatology* (NY) 2: 19 (1975)
3. Burtis, C.A. and Ashwood, E.R. (Eds), *Tietz Textbook of Clinical Chemistry*, 2nd edition, W.B. Saunders CO., Philadelphia (1994).
4. Data on file at Medica.
5. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
6. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EasyRA Assay Parameters (DBIL)

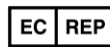
Primary Wavelength (nm)	550
Secondary Wavelength (nm)	700
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Yes (with each calibration)
Sample Blank	Yes
Reaction Time	4.5 min
Calibration interval (maximum)	28 days
Reagent on-board stability	30 days

Serum/Plasma

Sample volume (µl)	10.0
Diluent 1 volume (µl)	10
Diluent 2 volume (µl)	10
Reagent volume R1 (µl)	160
Reagent volume R2 (µl)	40
Decimal Places(default)	2
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend linear range)
Linearity	0.06 to 10 mg/dL



Medica Corporation, 5 Oak Park Drive
Bedford, Massachusetts 01730-1413 USA



Emergo Europe, Westervoortsedijk 60
6827 AT Arnhem, The Netherlands