BILIRUBIN
Instruction for use

Ref.: 31

Intended use . End point reaction system for direct and total bilirubins determination in blood samples.

Professional use.

[For in vitro diagnostic use.]

Test principle . Bilirubin is measured by diazotization and red coloured azobilirubin formation with maximum absorbance in 525 nm. Direct bilirubin (diglycuronide) is measured in aquous medium, while the total (direct and indirect) is measured by the action of a potent solubilizer of catalytic action.

Summary . The Bilirubin Labtest system is a simple method, rapid and with high specificity for total and direct bilirubin determination in only 5 minutes. Accelerator reagent, containing benzoate and buffered caffeine, dissolves fast and provide diazotination of indirect bilirubin allowing to obtaining coloured and non-cloud solution. Sodium nitrate stabilization is a security factor during measurement, allowing the storage of all reagents at room temperature.

The high method versatility allows that values up to 25 mg/dL to be measured without serum dilution. Bilirubin determination of icteric blood from newborn child is possible due the method versatility.

The proposed method uses manual technique and is easily applied to most semi-automated equipments which are able to measure an end point reaction absorbance in a range of 500 and 540 nm.

Methodology . Sims-Horn.

Reagents

1. R 1 **- (Accelerator) - Store at 15 - 25 ºC.**
Reagent label bears expiration date. Caffeine (130 mmol/L); sodium benzoate (260 mmol/L); sodium acetate (460 mmol/L) and surfactant.

2. R 2 **- (Sulfanilic acid) - Store at 15 - 25 ºC.**
Reagent label bears expiration date. Sulfanilic acid (5.75 mmol/L) and stabilizer.

3. R 3 **- Sodium nitrate - Store at 15 - 25 ºC.**
Reagent label bears expiration date. Sodium nitrate (72.5 mmol/L) and stabilizer.

Precautions and warnings

The usual security cares should be applied on the reagent handling.

Keep the dropper exactly on vertical position while Diazo Reagent preparing.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. During handling, the reagents are subject to chemical and microbial contaminations that may lead to reduced stability.

Materials required not provided

1. Photometer capable of measuring absorbance at 500 - 540 nm.
2. Pipettes to measure reagents and samples.
3. Timer.

Sample

A Standard Operating Procedure (SOP) should be established to establish appropriate procedures for the collection, preparation and storage of the sample. We emphasize that the errors due to the sample may be much larger than the errors that occurred during the analytical procedure.

Use serum or plasma (Heparin, EDTA). Bilirubin is reportedly stable in serum for about 4 days at 2 - 8 ºC and 3 months at -20 ºC, when not exposed to light.

Hemolytic sera provide false decreased results of direct bilirubin.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Disposal of all waste material should be in accordance with local guidelines.

Interference

Hemoglobin up to 30 mg/dL and triglycerides up to 170 mg/dL do not interfere significantly.

Triglycerides ranging from 170 - 3500 mg/dL provide false decreased results.

Hemoglobin values ranging from 30 - 180 mg/dL provide false increased results of total bilirubin and false decreased results of direct bilirubin.

Procedure

See notes 1 and 2.

The procedure used for calibration and test must be the same.

Preparing the diazo reagent .
Add 0.01mL of sodium nitrate (nº 3) to 0.3 mL sulfanilic acid (nº 2). Mix and use in the same day.

Labtest

	Blank	Direct	Total
Reagent water	1.0 mL	1.0 mL	----
Accelerator (nº 1)	----	----	1.0 mL
Sulfanilic acid (nº 2)	0.1 mL	----	----
Diazo reagent	----	0.1 mL	0.1 mL
Sample	0.05 mL	0.05 mL	0.05 mL

Mix and wait 5 minutes. Determine the absorbance of direct and total bilirubin in 525 nm or green filter (500 - 540) against Blank. The color is stable during 30 minutes.

The suggested measurement procedures are suitable for photometers whose minimum solution volume for reading is equal to or less than 1.0 mL. The volume adjustment must be checked for the photometer used. The volumes of sample and reagent may be proportionally modified without prejudice to the performance of the test and the calculation procedure remains unchanged. In case of volume reduction it is essential to observe the minimum volume required for the photometric reading. Sample volumes less than 0.01 mL are critical for manual applications and should be used with caution because they increase the imprecision of the measurement.

Quality control . For quality control use Qualitrol Level 1 and Qualitrol Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective measures to be taken if values fall outside the control limits.

Calculations

$$\text{Bilirubin (mg/dL)} = \frac{A_{unknown}}{A_{standard}} \times 10$$

Due the great reproductive results of the assays system, it is possible to use the factor method:

$$\text{Calibration factor} = \frac{\text{Standard concentration (mg/dL)}}{A_{standard}}$$

$$\text{Bilirubin (mg/dL)} = A_{Unknown} \times \text{Factor}$$

Indirect bilirubin is obtained by the difference between total and direct bilirubin.

Measurement/reportable range

Up to 25.0 mg/dL .

If Bilirubin concentration exceeds 25.0 mg/dL the sample must be diluted with 0.85% NaCl. Multiply the result by the appropriate dilution factor.

Expected values . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Direct Bilirubin

Age	mg/dL
Newborn[5]	up to 0.4

Total Bilirubin

Newborn[5]	
Age	mg/dL
1 day	up to 5.1
1 - 2 days	up to 7.2
3 - 5 days	up to 10.3

Children, teenagers and adults (mg/dL)

Direct Bilirubin	up to 0.4
Total Bilirubin	1.2

Performance characteristics[7]

Recovery studies . In three samples with bilirubin concentrations of 1.15, 1.30 and 20.4 mg/dL were added different quantities of analyte. Subsequent analyses provided recoveries ranging from 100 to 108%. The mean proportional systematic error at 2.5 mg/dL decision level was 0.085 mg/dL or 3.4%.

Method comparison . A group of 40 sera were assayed by the proposed method and a similar technique. Serum Bilirubin values ranged from 0.19 - 21.1 mg/dL the comparisons yielded a correlation coefficient of 0.995 and regression equation was $y = 0.940x + 0.247$. The mean total systematic error (proportional and constant) at 2.5 mg/dL decision level was 0.097 mg/dL or 3.9 %.

Imprecision - Within Run

	N	Mean (mg/dL)	SD (mg/dL)	(%) CV
Sample 1	20	1.43	0.03	2.05
Sample 2	20	2.34	0.03	1.16
Sample 3	20	21.9	0.22	1.01

Imprecision - Run-to-Run

	N	Mean (mg/dL)	SD (mg/dL)	(%) CV
Sample 1	20	1.44	0.07	4.97
Sample 2	20	2.34	0.07	3.04
Sample 3	20	22.0	0.69	3.15

Analytical sensitivity . Detection limit: 0.06 mg/dL for total bilirubin and 0.03 mg/dL for direct bilirubin

The detection limit represents the lowest measurable bilirubin concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without bilirubin.

Effects of matrix dilution . Two samples with values equal to 18.7 and 19.5 mg/dL were used to evaluate the system response in the matrix dilutions with 150 mmol / L NaCl (0.85%). Using dilution factors ranging from 2 to 8, recoveries between 100 and 105% were found.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents and use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm or conductivity ≤1 microsiemens/cm and silicates concentration must be < 0.1 mg/L.

References

1. Malloy HT, Evelyn KA. J Biol Chem 1937; 119:481.

2. Martineck RG. Clin Chim Acta 1966; 13:161.

3. Sims FH, Horn C. Am J Clin Path. 1958;29:412.

4. Sociedad Española de Bioquimica Clínica y Patologia Molecular, Base de Datos de Variación Biológica. Disponível em : <http:www.seqc.es/article/articleview/330/1/170> (acesso em 08/2005).

5. Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.

6. Soldin SJ, Brugnara C, Wong EC: Pediatric Reference Ranges, 5a. edição, Washington:AACC Press, 2005:42-43.

7. Labtest: data on file

Presentation

Product	Reference	Contents	
Bilirubin	31	R 1	1 X 250 mL
		R 2	1 X 120 mL
		R 3	1 X 5 mL

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

Labtest Diagnóstica S.A.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

Conteúdo suficiente para < n > testes Contenido suficiente para < n > tests Contains sufficient for < n > tests	**Risco biológico** Riesgo biológico Biological risk
Data limite de utilização (aaaa-mm-dd ou mm/aaaa) Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	**Marca CE** Marcado CE CE Mark
Material Calibrador Material Calibrador Calibrator Material	**Tóxico** Tóxico Poison
Material Calibrador Material Calibrador Calibrator Material	**Reagente** Reactivo Reagent
Limite de temperatura (conservar a) Temperatura limite (conservar a) Temperature limitation (store at)	**Fabricado por** Elaborado por Manufactured by
Representante Autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	**Número do lote** Denominación de lote Batch code
Consultar instruções de uso Consultar instrucciones de uso Consult instructions for use	**Controle** Control Control
Número do catálogo Número de catálogo Catalog Number	**Controle negativo** Control negativo Negative control
Adições ou alterações significativas Cambios o suplementos significativos Significant additions or changes	**Controle positivo** Control positivo Positive control
Produto diagnóstico in vitro Dispositivo de diagnóstico in vitro In vitro diagnostic device	**Controle** Control Control
Liofilizado Liofilizado Lyophilized	**Corrosivo** Corrosivo Corrosive
Período após abertura Período post-abertura Period after-opening	**Uso veterinário** Uso veterinario Veterinary use
Instalar até Instalar hasta Install before	**Fabricado em** Elaborado en Manufactured on
Produto de uso único Producto de un solo uso Single use product	

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