BILIRUBIN (DIRECT)

Automated Systems

BioSystems

COD 12504 5 x 50 mL
Only for *in vitro* use in the clinical laboratory

BILIRUBIN (DIRECT)
DICHLOROPHENYL DIAZONIUM

INTENDED USE

Reagent for the measurement of direct bilirubin concentration in human serum or plasma. The obtained values are useful as an aid in the diagnosis and control of the evolution of the jaundice.

This reagent is for use in the BioSystems A25 and A15 analyzers or in other analyzer with similar performance characteristics.

CLINICAL SIGNIFICANCE

Bilirubin is a waste product derived from the heme moiety of the hemoglobin released from senescent or damaged erythrocytes, that are destroyed in the reticuloendothelial cells. After production, bilirubin is transported to the liver in association with albumin. Inside the hepatocytes bilirubin is conjugated with glucuronic acid and it is excreted into bile. A number of inherited and acquired diseases affect production, uptake, metabolism, and excretion of bilirubin, resulting in hyperbilirubinemia[1,2].

Unconjugated hyperbilirubinemia is seen in newborns (physiological jaundice), in increased red cell destruction (hemolytic anemia, extensive hematoma), in ineffective erythropoiesis and in some rare genetic diseases (Gilbert's syndrome, Crigler-Najjar syndrome).

Conjugated hyperbilirubinemia is associated to a decreased excretion of bile due to liver diseases (hepatitis or cirrhosis) or to intrahepatic or extrahepatic cholestasis.

Jaundice is a clinical manifestation of hyperbilirubinemia, consisting of deposition of bile pigments in the skin, resulting in a yellowish staining of the skin and mucous membranes.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

Direct bilirubin in the sample reacts with 3.5-dichlorophenyl diazonium salt forming a coloured complex that can be measured by spectrophotometry at 535 nm[3]. Both direct and indirect bilirubin couple with diazo in the presence of cetrimide[4,5]. The terms "direct" and "total" refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilizing (accelerating) reagents. The "direct" and "indirect" bilirubin are only approximately equivalent to the conjugated and unconjugated fractions.

CONTENTS AND COMPOSITION

A. Reagent: 5 x 40 mL. Phosphoric acid 90 mmol/L, HEDTA 4.5 mmol/L, sodium chloride 50 mmol/L, pH 1.5.

DANGER: H314: Causes severe skin burns and eye damage. P260: Do not breathe dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

B. Reagent: 5 x 10 mL. 3.5-dichlorophenyl diazonium 1.5 mmol/L.

DANGER: H314: Causes severe skin burns and eye damage. P260: Do not breathe dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are stored at the recommended temperature, well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 2 months.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044.

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines.

REAGENT PREPARATION

Reagents are provided ready to use.

SAMPLES

Serum and plasma collected by standard procedures. Heparin or EDTA may be used as anticoagulants.

Bilirubin concentration in serum and plasma is stable for 2 days at 20-25ºC, 7 days at 4-8ºC and 6 months at −20ºC if protected from light[6].

CALIBRATION

A reagent blank should be done every day and a calibration at least every 2 months, after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007. 18010 and 18043) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

REFERENCE VALUES

Adults[7]:

Direct:	Up to 0.4 mg/dL = 6.8 µmol/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer and following the guidelines of the Clinical & Laboratory Standards Institute (CLSI). Results are similar with A15.

- Detection limit: 0.07 mg/dL = 1.15 µmol/L. Quantification limit: 0.37 mg/dL = 6.32 µmol/L.
- Linearity limit: 15 mg/dL = 257 µmol/L.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
0.492 mg/dL = 8.42 µmol/L	5.8 %	8.4 %
1.09 mg/dL = 18.6 µmol/L	2.8 %	6.3 %
2.01 mg/dL = 34.4 µmol/L	2.1 %	5.4 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: lipemia (triglycerides 1300 mg/dL) and hemolysis (hemoglobin 25 mg/dL) interfere. Other drugs and substances may interfere[8].
- Occasionally, somewhat higher values for direct bilirubin than for total bilirubin may be yielded by samples in which the total and direct bilirubin concentrations are similar.

BIBLIOGRAPHY

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3. Thaler M, Luppa PB and Schlebusch H. Bilirubin measurement – an updated survey. J Lab Med 2008; 32:1-9.
4. Zoppi F, Peracino A, Fenili D, Marcovina S and Ramella C. Metodo per la determinazione della bilirubina totale e coniugata. Uso di un tensioattivo cationico come agente solubilizzante. Giorn It Chim Cl 1976; 1:343-359.
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TEST PARAMETERS

R1: use Reagent A.
R2: use Reagent B.

	A25	A15
GENERAL		
Name	**BILIRUBIN DIRECT**	**BILIRUBIN DIRECT**
Sample type	serum / plasma	serum / plasma
Analysis mode	bireagent differential	bireagent differential
Units	mg/dL	mg/dL
Turbidimetry test	no	no
Decimals	2	2
Type of reaction	increasing	increasing
PROCEDURE		
Reading mode	bichromatic	bichromatic
Main filter	535	535
Reference filter	670	670
Sample	14	14
Vol. R1	240	240
Vol. R2	60	60
Washing	1.2	1.2
Reading 1 (cycle)	6	3
Reading 2 (cycle)	10	7
Reagent 2 (cycle)	7	5
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	multiple	multiple
Number of calibrators	1	1
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	0.04	0.04
Kinetic blank limit	-	-
Linearity limit	15	15
Substrate depletion	-	-

BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)
www.biosystems.global