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AZIENDA CERTIFICATA
UNI EN ISO 9001:2008
UNI EN ISO 13485:2004
CERTIFIED COMPANY

DIRECT BILIRUBIN

Colorimetric method with sample blank.

IVD CE In conformity with Directive 98/79 CE

000271 Rev.4 of 21/10/11

REF		
	ASR01033/1	**4x100 + 1x25 ml**
	ASR01033/2	**2x100 + 1x25 ml**
	ASKIT0302	**6x45 + 6x1,2 ml**

• Principle

Total bilirubin reacts in acid conditions with diazotized sulfanilic acid to form a red coloured azobilirubin.
The intensity of the produced colour is directly proportional to the amount of bilirubin present in the sample.
Direct bilirubin is glucoronic acid conjugated, water soluble and it reacts directly.
Total bilirubin is obtained by the presence of an accelerating agent which separates it from albumin.
Indirect bilirubin can be calculated by subtracting direct bilirubin from total bilirubin.

• Sample

Not hemolized fresh serum.
Samples must be analyzed within two hours from their collection and kept at room temperature and not exposed to light.
Direct Bilirubin (BIL-D) in serum is stable for 12 hours when stored in the refrigerator at 2 – 8°C and 3 months at – 20°C, if not exposed to sunlight.
Direct sunlight may cause up to 50% decrease in BIL-D within 1hour.
Shake and bring the samples at room temperature before using.

• Expected value

SERUM	< 0.35 mg/dl	< 6.0 µmol/l

Consider the above mentioned values as a reference.
It is strongly recommended that each laboratory establishes its own normal range according to its geographic area.

• Kit composition and possible risk classification

REAGENT (A)	
Sulfanilic Acid	< 1 %
Hydrochloric Acid 23%	< 5 %
REAGENT (B)	
Sodium Nitrite	< 1 %

The kit doesn't contain substance or prepared classified as dangerous according to the currently legislation.
The Reagent (A) contains sulphanilic acid that may produce an allergic reaction.

• Package: collection and storage

Store at temperature indicated upon the label.
Stable until the expiration date reported on the package.
After unsealing, it is advised to close up the bottle immediately in order to avoid evaporation, direct light exposure and bacteric contamination.

• Reagent preparation and stability

Liquid reagent ready to use, must be at room temperature (15 – 25°C) before using.
The Reagent is limpid and colourless.
Mono-reagent Procedure :
Add in the ratio 1:61 (0.5 ml + 30 ml) Reagents (B) and (A).
Stable at least 5 day at room temperature in dark bottle.
Double-reagent Procedure:
Liquid reagent ready to use.

• Precautions and warning

Whenever agents infettantis, chemical reagents, reagents of human/animal origin, blood or other biological liquids are manipulated, it is advisable to follow the most common recommendations and take all the necessary hygienic precautions as the monouse gloves.

• Waste disposal

Please consult local regulations for a correct waste disposal.

• Operative method

Wavelength:	555nm (546-578)
Cuvette:	1cm light path
Temperature:	+20/30/37°C.
Zero operation:	Against reagent blank
Assay type:	End Point
Sample/Reagent ratio:	1/15(monoreagent)

Mono-reagent Procedure:
Prepare two series of tubes, one for samples blank absorbance reading ,and one for samples absorbance reading.

Pipetting in tubes	*Reagent Blank*	*Sample/ calibrator*	*Samp/calbr* Blank
Reagent (A+B)	1500 µl	1500 µl	
Reagent (A)			1500 µl
Sample		100 µl	100 µl
Distilled Water	100 µl		

Double-reagent Procedure:
Prepare two series of tubes, one for samples blank absorbance reading ,and one for samples absorbance reading.

Pipetting in tubes	*Reagent Blank*	*Sample/ calibrator*	*Samp/calbr* Blank
Reagent (A)	1500 µl	1500 µl	1500 µl
Reagent (B)	25 µl	25 µl	
Sample		100 µl	100 µl
Distilled Water	100 µl		25 µl

Mix, incubate for exactly 5' at room temperature (15 – 25°C) and read samples/calibrator absorbance and samples/calibrator blank absorbance.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit.

• Calculation

FACTOR CALCULATION
BIL-D = (E) Sample(E) Calibrator blank x 12 a 546/578nm
CALIBRATOR CALCULATION

$$\text{BIL-D mg/dl} = \frac{\text{(E) Sample - (E) Sample blank}}{\text{(E) Calibrator - (E) Calibrator blank}} \times \text{Cal. Value}$$

Factor to convert mg/dl in µmol/l = 17.1.

• Performance

A. METHOD PERFORMANCE

Measure interval/linearity:	0.056 – 20 mg/dl
Detection limit (2DS):	0.0558 mg/dl
Sensitivity:	0.1 mg/dl = 0.00652A (546nm)

B. INTRA-ASSAY PRECISION (2DS): n=20

	Mean	C.V.(%)
Low control	M = 0.19 mg/dl	2.78 %
Medium control	M = 0.65 mg/dl	1.20 %
High control	M = 3.12 mg/dl	0.95 %

C. INTER-ASSAY PRECISION (2DS): n=20

	Mean	C.V.(%)
Low control	M = 0.20 mg/dl	5.12 %
Medium control	M = 0.66 mg/dl	1.52 %
High control	M = 3.16 mg/dl	1.27 %

D. CORRELATION BETWEEN METHODS
This method compared with a correspondent one from the competition, has given the following results:
N = 40 r = 0.998 y = 1.04 x + 0.07

E. INTERFERENCE (in accordance with recommendations SFBC)
1. Triglicerides don't interfere up to 2000 mg/dl
2. Hemoglobin does not interfere up to 500 mg/dl
For a thorough evaluation of the interfering substances, consult: Young, D.S.,et al.,Clin.Chem. 21:1D (1975).

• Limitations

For concentration higher than 20 mg/dl, repeat the measure on sample diluted 1:2 with saline solution and multiply the results by 2.

• Quality Control

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology. For this purpose the use of test serum REF. ASR02010 (Normal level) and REF. ASR02020 (Pathologic level) is suggested.

• Bibliography

Ehrlich, P., Zeitschr, Sur Anal. Che mie 23:275 (1884).
Vassault,A. et al. Ann.Biol.Clin.,44,686, (1986).