Direct Bilirubin – Method Diazo Acid

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182500	n° 20 vials x 10 mL (R.A) n° 1 vials x 15 mL (R.B)
CHEMILAB	REF B81180031	n° 3 vials x 28 mL (R.A) n° 1 vials x 1 mL (R.B)
	REF B81180032	n° 10 vials x 34 mL (R.A) n° 1 vials x 4 mL (R.B)

INTENDED USE

Product for use in the quantitative determination in vitro of the Direct Bilirubin concentration in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

The human organism produces daily 250-300 mg of bilirubin; about 85% of this amount comes from the heme fraction of hemoglobin, formed by the destruction of erythrocytes within the reticulo endothelial cells of the liver, spleen and bone marrow. The remaining 15% derives from erythrocyte precursors and from the catabolism of other proteins, e.g. myoglobin.

PRINCIPLE OF THE METHOD

Method colorimetric diazo acid. Sulphanilic acid reacts with sodium nitrite, forming sulphanilic acid diazotate. In the absence of detergents or accelerants, the direct bilirubin couples with the sulphanilic acid diazotate in the first minute, forming azobilirubin. The colour formed is directly proportional to the direct bilirubin present in the sample.

Storage and stability

= storage temperature 15-25°C

Stored closed at the indicated temperature avoiding direct light, evaporation and contamination of any kind, intact reagents are stable until the expiry date indicated on the label.

Concentrations

Reagent A:			
	Conc.	U.M.	*GHS05
Sulphanilic Acid	32.0	mmol/L	
Hydrochloric Acid	166	mmol/L	
Reagent B:			
Sodium Nitrite	29.0	mmol/L	

Signal word**: DANGER**

Contains sulphanilic acid (CAS 121-57-3): May produce an allergic reaction.

H314 - Causes severe skin burns and eye damage.

P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or 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.

P310 - Immediately call a POISON CENTER/doctor.

P321 - Specific treatment (see on this label).

P501 - Dispose of contents/container in accordance with local/ regional/ /international

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements.
Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

Procedure

Quality control

Control sera with a known titer of Direct Bilirubin are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration

For calibration use the "Calibrator serum Sclavo" Code B35181702.

Traceability

The Direct Bilirubin traceability is reported in the package insert supplied with the "Calibrator Serum."

SAMPLE

Type of sample and storage

Fresh, non-hemolyzed serum or plasma samples should be used. Store out of the light. The samples must be tested within 2 hours if stored at room temperature or within 12 hours if stored at 2 - 8°C. If frozen between - 15°and - 20°C, the samples are stable for 3 - 4 months.

PREPARATION OF THE REAGENT

Add 1 volume of Reagent B to a 100 volume of Reagent A and mix gently. The reagent is thus ready for use. The reagents are stable for 25 days if closed and stored at 2 - 8°C. The reagent must be kept carefully out of the light. Slight variations in colour among batches will not affect test results.

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	540 nm
Temperature:	37°C
Reaction	Endpoint (Increasing reaction)

Technique – Monoreactive method

Bring the reagents to rection temperature and operate away from direct light.

	U.M.	Blank	Calib. serum	Sample
Reagent A+B	µL	1000	1000	1000
Calib. Serum	µL	-	50	-
sample	µL	-	-	50
Blank	µL	50	-	-

Mix well and let stand for 2 minutes at 37°C.

Reading

Read at 540 nm the sample and calibrator serum absorbance against blank reagent. Final colour is stable for 1 hour avoiding direct sunlight.

Results:

Manual Method

Calculation of Direct Bilirubin concentration:

$$\frac{\text{O.D Sample}}{\text{O.D. Calibr. Serum}} \times \text{Concen. Calibr. Serum} = \text{Direct Bilirubin mg/dL}$$

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Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

REFERENCE RANGE
Serum or plasma:
Up to < 0,2 mg/dL (3,4 µmol/L).
Each laboratory must establish its own normal-range values on the basis of its population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
The method is linear up to 15 mg/dL of Direct Bilirubin. If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Accuracy
Commercial control sera were analyzed with the Direct Bilirubin kit following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Mean	DS	CV%	Recovery
Low	5	0.20	0.000	0.00	111%
High	5	1.70	0.055	2.16	91.4%

Interferences

Interference	Limits
Haemoglobin	**400 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	0.76	0.02	2.63	30
High	mg/dL	2.24	0.05	2.23	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	0.71	0.019	2.68	20
High	mg/dL	2.18	0.051	2.34	20

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 0.01 mg/dL at the conditions established for this test.

Comparison between methods
The method was compared with a similar commercially available method, analyzing 61 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-0.19
Slope	1.063
Correlation Coeff. (R)	0.99

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCES

1 **Thomas L. (1998).** Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft;. p. 208-14.
2 **Newman DJ, Price CP**. (1999) Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. Tietz ext book of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; p. 1204-70.
3 **Guder WG, Zawta B et al.** The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001.p. 48-9, 52-3.
4 **Newman JD, Price PC (1999).** Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia:W.B Saunders Company; p. 1250.
5 **Clinical Laboratory Standards Institute (CLSI).** User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition. EP15-A2.
6 **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2.
7 **Clinical Laboratory Standards Institute (CLSI).** Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3.
8 **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17
9 **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07.
10 **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition - EP06.

REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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