Iron - method Iron "Ferene S"
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182529	n° 10 vials x 20 mL (R.A) n° 1 vials x 11 mL (R.B) n° 10 vials x 1 mL (R.C lio)

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

CLINICAL SIGNIFICANCE
Iron is present in body fluids as a component of haemoglobin and myoglobin. It is transported in the plasma by a protein, transferrin. Ferritin constitutes the plasma reserve of iron and allows regulation of the plasmatic concentration. High increases in plasma iron values are an indication of hemochromatosis and liver damage. Low iron levels may be caused by anemia due to malabsorption because of gastrointestinal disease or to significant losses through menstruation. In order to control iron metabolism and obtain more detailed information it is advisable to determine the transferrin and ferritin concentrations.

PRINCIPLE OF THE METHOD
Method Iron "Ferene S". In the presence of a buffer system (pH 4.5), the iron is first freed by transferrin and then reduced by ascorbic acid to the ferrous state. The iron^{2+} thus obtained forms a stable-coloured compound with the specific "Ferene S" complexing agent. The intensity of the colour formed, read at λ 600 nm, is proportional to the amount of iron present in the test sample.

$$(Fe^{3+} - \text{Transferrina}) + (\text{Tampone} - \text{Tiourea}) \xrightarrow{pH4,5} Fe^{3+} + \text{Transferrina}$$

$$Fe^{3+} + \text{Ac. Ascorbico} \xrightarrow{\text{Riducente}} Fe^{2+} + \text{Complessante} \xrightarrow{\text{FereneS}} \text{Comp. Colorato}$$

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

(Referred to the reagent ready for use)

Reagent A:			
	Conc.	**U.M.**	
Acetate buffer (pH 4,5)	1.30	mmol/L	*GHS02 / GHS05
Thiourea	25.0	mmol/L	
Tensioactive agent	5.00	g/L	
Guanidine hydrochloride	>4,0	M	
Reagent B:			
Ferene-S	>20.0	mmol/L	
Reagent C:			
Ascorbic acid	17.0	mmol/L	

* Warning: **DANGER -** *Contains Acetic Acid (CAS 64-19-7) – Sodium Hydroxyde (CAS 1310-73-2)*
H226 - Flammable liquid and vapour.
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/national/international regulations.

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 Iron 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 Iron traceability is reported in the package insert supplied with the calibrator serum.

SAMPLE
Type of sample and storage.
Serum and plasma samples should be used. Do not use haemolysed samples.
Samples can be stored for 7 days at 25°C or 1 month at 4°C.

REAGENT PREPARATIONS
Slight variations in color from batch to batch, will not affect test results.
Procedure Bireagent
Reagent A (A + C) preparation: dissolve the content of the vial of Reagent C with the content of a vial of Reagent A. Mix to a complete dissolution.
Reagent B: (chromogen Ferene-S) ready to use liquid reagent.
This reagent is ready for use and stable for 30 days if kept at 2-8°C away from direct light.
Procedure Monoreagent
Reagent A (A + C) preparation: dissolve the content of a vial of Reagent C with the content of a vial of Reagent A. Mix to a complete dissolution.
Add. 1 volume of reagent B to 20 volumes of Reagent A (Reagent A + Reagent C).
After reconstitution, the reagent is stable up to 30 days if closed and kept at 2-8°C.

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 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):	600 nm
Temperature:	37°C
Reaction:	End-point (increasing reaction)

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Technique – Bireagent
Bring the reagents to reaction temperature and operate away from direct light.

	U.M.	Blank	Calibrator serum	Sample
Reagent 1 (A+C)	µL	1000	1000	1000
Sample	µL	-	-	100
Blank	µL	100	-	-
Calibrator serum	µL	-	100	-
Mix and after about 5 minutes, read the absorbance sample blanks and add:				
Reagent 2 (B)	µL	70	70	70

Mix and after about 5 minutes, read at 600 nm the absorbance of sample, standard and reagent blank. The final colour is stable for 1 hour.

Technique – procedure with Serum as starter

	U.M.	Samp. Blank	Blank	Calib. serum	Sample
Reagent A	µL	1000	-	-	-
Working Reag. (A+B) +C	µL	-	1000	1000	1000
Blank	µL	-	100	-	-
Calibrator serum	µL	-	-	100	-
Sample	µL	100	-	-	100

Mix and after about 5 minutes, read at 600 nm the absorbance of sample blank, samples, standard and reagent blank. The final colour is stable for 1 hour.
The reaction volumes can be varied proportionally without any alteration of the results.

Results:
Manual Method
Subtract the absorbance of its own sample blank and reagent blank from the absorbance of each sample:
O.D. Sample = O.D. Sample – (O.D. Sample Blank + O.D. Reagent Blank)
O.D. Calibrator Serum = O.D. Calibrator Serum – O.D. Reagent Blank
Calculation of Ferro Ferene concentration:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator serum}} \times \text{Calibrator Serum Concentration} = \mu\text{g/dL of Iron}$$

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 single instruments.

REFERENCE RANGE
Serum and Plasma:
- Males: 65 - 175 µg/dL (11,6 - 31,3 µmol/L)
- Females: 50 - 170 µg/dL (9,00 – 30,4 µmol/L)

Each laboratory must establish its own normal-range values based on its population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
- Mono-Reagent Method: the reaction is linear up to 1032 µg/dl (184.8 µmol/L)
- Two-Reagent Method: the reaction is linear up to 986.7 µg/dl (176.7 µmol/L)

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

Range	Replicates	Mean (µg/dL)	DS	CV%	Recovery
Low	5	69,4	2,408	3,47	94,2 %
High	5	226,4	1,817	0,80	97,2 %

Interferences

Interference	Limits
Triglycerides	**300 mg/dL**
Bilirubin	**58 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	g/dL	237	3.62	1.52	30
High	g/dL	67.9	1.38	2.04	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	g/dL	229	1.89	0.82	20
High	g/dL	65.2	1.02	1.57	20

Limit of Sensitivity
Mono-Reagent Method: At λ 600 nm, an absorbance of 0.008 corresponds to a concentration of about 10.0 µg/dL of Iron.
Two-Reagent Method: At λ 600 nm, an absorbance of 0.026 corresponds to a concentration of about 11.0 µg/dL of Iron.

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	-4.403
Correlation Coeff. (R)	0.991

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

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

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