

UIBC Reagent

Liquid FerroZine® Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8 °C
Linear Range	:	Up to 89 µmol/L (500 µg/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

INTENDED USE

This reagent is intended for the in vitro quantitative determination of UIBC in human serum.

CLINICAL SIGNIFICANCE

Unsaturated Iron-binding capacity (UIBC) measurements are used to assist in the diagnosis and treatment of anaemia.

METHODOLOGY

Stookey¹ and Persijn² reported that Ferrozine, a sulfonated derivative of diphenyltriazine, was a more sensitive spectrophotometric reagent for UIBC reagent. The technique avoids protein precipitation and minimizes interference from other trace materials.

At alkaline pH, a known excess of ferrous ions added to serum bind specifically with available iron-binding sites of transferrin, saturating the molecules with iron. Ferrozine then reacts with the remaining unbound iron to form a strongly coloured purple complex measured at 560 nm. The difference between the known excess amount of iron added and the remaining unbound iron is equivalent to the unsaturated iron-binding capacity (UIBC). Total iron-binding capacity (TIBC) may be calculated as serum iron plus UIBC.

In addition to iron, copper is the only other trace metal found in serum which reacts with Ferrozine to form a coloured complex. Neocuproine is therefore also present in the colour reagent to prevent copper interference.

REAGENTS COMPOSITION

Active Ingredients

	<u>Concentration</u>
Reagent B: Chromogen	
Ferrozine	7.8 mmol/L
Hydroxylamine hydrochloride	0.22 mol/L
Neocuproine	14.4 mmol/L
Reagent C: UIBC buffer	
Tris (hydroxymethyl)	0.5 mol/L
Aminomethane, pH 8.1 (25 °C)	
Also contains preservative and surfactant.	
Reagent D: Saturating Standard	
Iron	89 µmol/L
(as ferrous ammonium sulphate)	(500 µg/dL)
Hydroxylamine hydrochloride	0.72 mol/L

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. For further information consult the UIBC Reagent Material Safety Data Sheet.

Reagent B and Reagent D:

R43	May cause sensitisation by skin contact.
S24	Avoid contact with skin.
S37	Wear suitable gloves.

REAGENT PREPARATION

Reagent B, Reagent C and Reagent D are ready to use as supplied.

STABILITY AND STORAGE

Prior to use:

When stored refrigerated at 2-8 °C and protected from light, the reagents are stable until the expiry date stated on the bottle and kit box label.

Once the Reagent is Opened:

When stored capped at 2-8 °C the reagents are stable until expiry.

Indications of Reagent Deterioration:

- Turbidity,
- Presence of precipitate; and/or
- Failure to recover control values within the assigned range.

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		Xi - Irritant
	Reagent B (Chromogen)		Reagent D (Saturating Standard)
	Reagent C (Buffer)		

SPECIMEN COLLECTION AND HANDLING

Collection: Blood should be collected using materials (e.g., syringes, test tubes) that are iron-free.

Serum: Use non-haemolysed serum.

Storage: Specimens are stable for at least 4 days at room temperature (18-25 °C) or one week at 2-8 °C³

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature, measuring absorbance at 560nm and accommodating a 3 reagent assay system.
- Analyser specific consumables e.g. sample cups.
- Calibrator or a suitable aqueous Iron standard.
- Normal and abnormal assayed control material.
- Distilled or deionised water and related equipment, eg. pipettes.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37 °C
Wavelength	560 nm
Assay Type	Endpoint
Direction	Increase
Sample:Reagent ratio	1:6
e.g. Sample vol	0.20 mL
Reagent C vol	1.00 mL
Reagent D vol	0.20 mL
Incubation Time	3 minutes
Reagent B vol	0.040 mL
Delay Time	10 minutes

MANUAL PROCEDURE

The following instructions are designed for manual instrumentation.

1. Label one test tube or cuvette for a reagent blank, each standard, control and unknown sample.
2. Add 1.0 mL of UIBC Buffer (Reagent C) to each tube or cuvette.
3. Add 0.4 mL of water to reagent blank tube. Add 0.2 mL of water and 0.2 mL of Saturating Standard (Reagent D) to the Standard tube. Add 0.2 mL of sample (control, unknown) and 0.2 mL of Saturating Standard (Reagent D) to appropriate tubes or cuvettes, mix and incubate for 5 minutes.
4. Zero spectrophotometer against reagent blank.
5. Read and record absorbance of each tube to obtain sample blanks (A1).
6. Add 0.040 mL of Chromogen (Reagent B) to all tubes, mix and incubate for 10 minutes.
7. Re-zero spectrophotometer against reagent blank with Chromogen (Reagent B) added.
8. Read and record absorbance of each tube to obtain test readings (A2).
9. Subtract the sample blank reading from the test reading to obtain ΔAbsorbance (A2 - A1).

CALCULATIONS

Results for UIBC and TIBC are calculated stepwise as follows:

$$\text{Excess Iron } (\mu\text{mol/L}) = \frac{\Delta\text{Absorbance of Unknown}}{\Delta\text{Absorbance of Standard}} \times \text{Conc of Std } (\mu\text{mol/L})$$

$$\text{UIBC } (\mu\text{mol/L}) = 89 (\mu\text{mol/L Iron added}) - \text{Excess Iron } (\mu\text{mol/L})$$

$$\text{TIBC } (\mu\text{mol/L}) = \text{Serum Iron } (\mu\text{mol/L}) + \text{UIBC } (\mu\text{mol/L})$$

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The colour development is stable for 30 minutes.
3. Even though the reaction of Iron with Ferrozine in fresh serum is instantaneous, in some lyophilized control sera the reaction is delayed and a 10 minute incubation is recommended.
4. Unit conversion: $\mu\text{mol/L} \times 5.585 = \mu\text{g/dL}$.

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturer's specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The Lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control should be run as unknown samples:-

- At least every eight hours
- When a new bottle of reagent is used
- After preventative maintenance is performed or a critical component is replaced
- With every calibration

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test
- If results remain out of control contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin, lipaemia and ascorbic acid were carried out and the following results were obtained:

Haemoglobin: Avoid the use of haemolysed samples.

Bilirubin: No interference from bilirubin up to 1026 $\mu\text{mol/L}$ (60 mg/dL).

Lipaemia: No interference from lipaemia, measured as triglycerides, up to 8.1 mmol/L (710 mg/dL).

Ascorbic Acid: No interference from ascorbic acid up to 10 mg/dL.

2. For a more comprehensive review of factors affecting UIBC assays refer to the publications of Young⁴, Martin⁵, and Constantino.⁶

EXPECTED VALUES⁷

TIBC	Adult Males:	44.8 - 80.6 $\mu\text{mol/L}$ (250 - 450 $\mu\text{g/dL}$)
	Adult Females:	44.8 - 80.6 $\mu\text{mol/L}$ (250 - 450 $\mu\text{g/dL}$)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

PERFORMANCE DATA

The following data was obtained using the Liquid UIBC Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁸

	Level I	Level II
Number of samples	20	20
Mean ($\mu\text{mol/L}$)	34.3	56.2
Mean ($\mu\text{g/dL}$)	191.7	313.7
CV (%) Within run	1.0	0.7
CV (%) Between day	1.8	5.0

ACCURACY

Comparison studies were carried out using a similar commercially available UIBC Reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of sample pairs	50
Range of sample results	24.7-86.3 $\mu\text{mol/L}$ (138-482 $\mu\text{g/dL}$)
Mean of reference method results	51 $\mu\text{mol/L}$ (284 $\mu\text{g/dL}$)
Mean of UIBC results	50 $\mu\text{mol/L}$ (281 $\mu\text{g/dL}$)
Slope	1.003
Intercept	-0.7 $\mu\text{mol/L}$ (-3.7 $\mu\text{g/dL}$)
Correlation coefficient	0.9698

LINEARITY

When run as recommended, the assay is linear up to 89 $\mu\text{mol/L}$ (500 $\mu\text{g/dL}$).

Linearity on various automated instruments may vary from this value. The user should consult the specific instrument application for the instrument specific linearity claim.

SENSITIVITY

When run as recommended, the sensitivity of this assay is 3.4 ΔmAbs per $\mu\text{mol/L}$ or 0.61 ΔmAbs per $\mu\text{g/dL}$ (1cm light path, 550nm).

REFERENCES

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REF

Reorder Information

REAG B REAG C REAG D

TR46201 1 x 14 mL 2 x 125 mL 1 x 50 mL