IVD CAPSULE Ferritin

REF P02.00024

English

Intended use

The **IVD CAPSULE Ferritin** is a single use, rapid in vitro diagnostic test for the quantitative measurement of ferritin in lithium heparin anticoagulated human capillary and venous whole blood as an aid in the diagnosis of iron deficiency in adults.

The **IVD CAPSULE Ferritin** is to be used with the **abioSCOPE 2.0** in vitro diagnostic test system. The system is intended for professional use in clinical laboratory settings or point of care (PoC) locations including near-patient testing.

Summary

One quarter of the world's population is thought to be iron deficient. [1] Iron deficiency is recognized to cause symptoms such as unexplained fatigue, mood disorders, restless leg syndrome and pallor, symptoms that result in impaired quality of life and working capacity.

Ferritin is a high-molecular weight protein made of 24 subunits that assemble into a spherical protein shell capable of storing iron. [2] The determination of blood ferritin concentration is the most sensitive and specific indicator of iron deficiency at an early stage.

However, as ferritin is an acute phase protein, its blood concentration increases independently of the iron stock in case of chronic inflammation, infection and other less frequent disorders. Some genetic disorders have also been associated with high ferritin concentration and therefore the clinical diagnosis of iron deficiency shall always be made in regards of the entire clinical evaluation of the patient. [3]

Test principle

The blood sample is mixed with a solution composed of fluorescently labeled antibodies reactive to human ferritin. The blood sample, now containing the ferritin-antibody complex, is loaded onto the capsule, then is drawn through the capsule by capillary action and passes through a built-in separator that excludes particles from the measurement area. After passing through the separator, the ferritin-antibody complex is bound by capture antibodies immobilized within the read-out area of the capsule.

The concentration of the captured ferritin is proportional to the fluorescence generated by the fluorophore conjugated to the signal antibody. The measured fluorescence signal is translated into ferritin concentration and expressed in ng/ml.

Reagents

Each assay contains one vial containing 150 µl of the **abioMIX** reagent. The **abioMIX** reagent is composed of the fluorescently labeled anti-human ferritin antibody, dissolved in Tris Buffered Saline solution supplemented with NaCl, KCl and Tween-20. The **abioMIX** reagent contains a preservative (Table 1).

Material	Concentration
Fluorescently labelled anti-human ferritin antibody	3 µg/ml
NaCl	0.137 M
KCl	0.0027 M
Tris Base	0.0248 M
Tween 20 (CAS number 005-64-5)	1% (v/v)
ProClin300 (CAS number 55965-84-9)	0.04% (v/v)

Table 1| Composition of the **abioMIX** reagent.

Materials include:

- 1x ferritin capsule
- 1x vial of **abioMIX** reagent
- 1x capillary blood collector
- 1x desiccant bag

Test procedures

Put the patient in an adequate position to perform the blood collection.

1. Clean the blood collection site with an alcohol pad (not supplied) using standard blood-draw procedures. Allow the collection site to dry completely.
2. Using the provided capillary blood collector, collect 50 µl of blood from the patient. If the blood is drawn from the fingertip, ensure filling of the capillary blood collector to the 50 µl mark.
3. Transfer the blood sample into the **abioMIX** vial. Dispense the blood sample into the **abioMIX** reagent to ensure mixing.
4. Close the vial and shake by flicking the vial or by tapping on a surface for at least 10 seconds to mix. A mixed reagent will have a uniform color.
5. Tap the **abioMIX** vial on a hard surface several times. This eliminates bubbles and knocks fluid droplets to the bottom of the vial.
6. Open the **abioMIX** vial. Insert the blood collection pipette into the solution. Allow capillary action to draw the mixed sample into the capillary tube.

7. Use the capillary tube to deposit the sample into the center of the measurement capsule. The sample should be dispensed slowly to allow solution to wick into the capsule.
8. Close the capsule. The lid folds over the top and snaps into place.
9. Place the capsule on the **abioSCOPE 2.0** loading tray.

To measure the sample, refer to the **abioSCOPE 2.0 User Manual**.

Storage and stability

Stored at 2-8 °C until the expiration date printed on the pouch. The **abioMIX** reagent is ready-to-use. Allow the **abioMIX** reagent to warm up to room temperature before use. Use **abioMIX** reagent within 4 hours of warming.

The blood sample mixed with the **abioMIX** reagent should be immediately loaded into the ferritin capsule. The filled capsule should be immediately measured.

Sample volume: 50 µl

Traceability and Calibration

The **IVD CAPSULE Ferritin** is calibrated by the manufacturer using a preparation of human liver ferritin in a serum base supplemented with red blood cells. The instrument automatically reads in the lot-specific calibration data that are embedded within the capsule chip, eliminating the need for calibration by the user. External calibration is not needed.

The **IVD CAPSULE Ferritin** is calibrated with a calibrator traceable to the 3rd Ferritin WHO International Standard, preparation 94/572.[4]

Quality control

For quality control, use the **IVD CAPSULE Ferritin Control**. Follow the applicable government regulations and local guidelines for quality control.

The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined target range. Each laboratory should establish corrective measures to be taken if values fall outside of the defined ranges.

Warnings and precautions

- For in vitro diagnostic use.
- The **IVD CAPSULE Ferritin** must be kept refrigerated until use.
- Do not freeze.
- Allow the **abioMIX** reagent vial to reach room temperature before use.
- The **IVD CAPSULE Ferritin** should be used within 4 hours after being removed from refrigeration.
- Use the provided capillary blood collector for blood draw.
- This product requires the handling of human specimens. It is recommended that all human-sourced material should be considered potentially infectious. Universal precautions that apply to your facility should be used for handling and disposal of materials during and after testing.[5]
- Do not use reagents after the expiration date printed on the box.
- The impact of hematocrits on test results has been evaluated in the range 30 to 50%. The impact of lower or higher hematocrit level is unknown.

Reagent deterioration

The following observations indicate reagent deterioration:

- Presence of turbidity in the **abioMIX** vial.
- Consistently high or low values from assay kits from the same batch.

Limitations

- This assay uses antibodies of murine (mouse) origin. Patient material reactive to mouse proteins may give anomalous results. Heterophilic antibodies in human blood can interfere with in vitro immunoassays. Patients routinely exposed to animals or to animal blood products can be prone to this interference. The impact of heterophilic antibodies and of human anti-mouse antibodies (HAMA) on measurements have not been assessed.
- Grossly hemolytic, icteric or grossly lipemic specimen should not be analyzed.
- High level (> 30 g/l) of immunoglobulins of type G has been shown to negatively bias test results of more than 20%.
- All assay materials are single-use and cannot be re-used.
- Diagnostics results should be interpreted within the complete clinical picture. Definitive diagnosis and/or clinical decision should not be based solely on the results of any single diagnostic test but made after all clinical and laboratory findings are evaluated.

Expected values

The normal range in serum ferritin concentration [ng/ml] of adults varies by sex and age group. [6]

Women

Age range (years)	n	Median	P5	P95
18-24	96	30	5	73
25-34	226	38	5	95
35-44	221	38	5	108
45-54	177	60	5	217
55-64	162	74	12	199
65-74	138	91	7	231
75+	99	77	6	209
Total	1119	56	5	170

Men

Age range (years)	n	Median	P5	P95
18-24	107	80	15	223
25-34	211	108	21	291
35-44	202	120	21	328
45-54	166	139	21	395
55-64	140	143	22	349
65-74	127	140	12	374
75+	80	110	10	309
Total	1033	121	16	328

N = number (sample size); P = percentile. Ferritin values are expressed in ng/ml.

It is recommended that each laboratory establishes its own expected reference range for the population it serves.

Ferritin levels below 30 ng/ml have been reported as indicative of iron deficiency anemia. [7] Lower cutoff values can also be found but are associated with a lower clinical sensitivity. Iron deficiency anemia can also be observed in patients with normal or elevated ferritin levels because of confounding factors, such as hepatocellular disease or iron therapy.

Measuring range: 20.0 - 600 ng/ml

The linearity of the assay was determined by diluting a pool of samples with clinically elevated ferritin level in a ferritin-depleted sample to concentrations spanning the entire anticipated assay range, with the hematocrit fixed at 43%. Regression analysis demonstrated that the assay response was linear with an R^2 value of 0.997 from 21.0 ng/ml to 830 ng/ml. The equation of the regression line with its 95% confidence intervals was y = 1.06 (0.99 to 1.13)x – 18.9 (-46.7 to 8.9).

Sample type comparability

The comparability of test results when the **IVD CAPSULE Ferritin** is used with lithium heparin anticoagulated capillary or venous whole blood, or venous plasma has been evaluated with several paired samples from volunteer donors whose ferritin concentrations span the entire assay reportable range. Table 2 summarized the findings of this study.

	Capillary versus venous whole blood (n = 13)	Venous whole blood versus venous plasma (n = 41)
Non-Weighted Deming linear regression		
Slope (95% CI)	1.12 (1.03 to 1.21)	1.07 (0.93 to 1.20)
Intercept (95% CI)	-5.7 (-25.6 to 14.2)	-8.1 (-21.5 to 5.3)
Least square linear regression		
Slope (95% CI)	1.10 (0.97 to 1.22)	1.04 (0.96 to 1.11)
Intercept (95% CI)	-2.6 (-27.9 to 22.7)	-4.3 (-17.6 to 9.0)
R^2	0.97	0.95

Table 2| Comparison of test results obtained with different sample types. Linear regression statistics were applied to the entire data set and correlations were expressed as the R^2 coefficient based on the least square regression line.

Relative biases with their 95% confidence intervals were also determined at three ferritin levels, namely 20, 50 and 150 ng/ml for both sample types comparison (Table 3).

	Bias at the following concentration of ferritin [ng/ml]		
	20	100	150
	Capillary versus venous whole blood		
Bias [ng/ml]	-3.3	0.2	11.9
Lower 95% CI	-22.2	-15.7	2.3
Upper 95% CI	15.5	16.0	21.4
	Venous plasma versus whole blood		
Bias [ng/ml]	-6.8	-4.8	1.8
Lower 95% CI	-65.2	-57.5	-43.0
Upper 95% CI	51.6	47.9	46.6

Table 3| Estimated biases at three ferritin levels with their 95% confidence intervals.

Precision

Various precision variance components of the **IVD CAPSULE Ferritin** have been determined. The between-day, -lot, -devices and -user imprecision had at all tested level (one < 100 ng/ml, one in the range 100 to 200 and one

> 400 ng/ml) %CV < 10 (except the low positive sample on the lot-to-lot who had imprecision above 10%). Repeatability of lithium heparin anticoagulated venous whole blood samples have been evaluated with 10 clinical samples covering the range of ferritin values 27.3 to 449 ng/ml. All samples were measured 10 times in a row. Imprecision values ranged from 11.6 to 34.1%, with higher imprecision observed in the low positive samples.

Interferences

Test results are not biased by more than 20% with the following substances up to a clinically elevated concentration of:
Free and conjugated bilirubin: 40 mg/dl
Hemoglobin: 1000 mg/dl
Triglycerides: 1500 mg/dl
Rheumatoid factor: 100 UI/ml
Biotin: 0.35 mg/dl

No high dose effect has been observed up to the highest tested ferritin concentration of 34000 ng/ml.

Method comparison

The **IVD CAPSULE Ferritin** on the **abioSCOPE 2.0** demonstrated a good comparability with two reference laboratory methods (ARCHITECT i2000SR Ferritin assay, Abbott Laboratories; and Roche Diagnostics Cobas 8000 Tina-quant 4th gen ferritin assay). 41 paired samples (lithium heparin venous whole blood on **abioSCOPE 2.0** and corresponding venous plasma on the two reference methods) were assessed on both methods in duplicates. The Table 4 summarizes the study results.

	abioSCOPE 2.0 versus Architect i2000SR	**abioSCOPE 2.0 versus Cobas 8000**
Non-Weighted Deming linear regression		
Slope (95% CI)	1.29 (1.08 to 1.50)	1.02 (0.86 to 1.19)
Intercept (95% CI)	2.9 (-15.2 to 21.1)	-3.8 (-23.7 to 16.2)
Least square linear regression		
Slope (95% CI)	1.20 (1.07 to 1.34)	0.96 (0.84 to 1.08)
Intercept (95% CI)	13 (-7 to 32)	5.5 (-15.4 to 26.3)
r (95% CI)	0.94 (0.89 to 0.97)	0.94 (0.88 to 0.97)
R^2	0.89	0.88

Table 4 | Comparison of methods Linear regression statistics were applied to the entire data set covering a range of value (on the **abioSCOPE 2.0**) of 19.6 to 429 (n = 41).

References

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4. WHO International Standard Ferritin, human, recombinant, NIBSC code: 94/572.
5. Clinical and Laboratory Standards Institute (CLSI), "Protection of Laboratory Workers From Occasionally Acquired Infections; Approved Guideline – Fourth Edition." CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
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CE marking

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LiH — Indicates that a lithium heparin anticoagulated whole blood sample must be used.