FERRITIN

| OSR61203 | 4 x 24 mL | R1 |
| | 4 x 12 mL | R2 |

Intended Use
The Ferritin Reagent is for the determination of ferritin concentrations in human serum and plasma on the Beckman Coulter AU clinical chemistry analyzers.

Summary
Serum ferritin is an indicator of body iron stores: it has been shown to correlate with stainable bone marrow iron. Measurements of ferritin aid in the diagnosis of diseases affecting iron metabolism, such as hemochromatosis (iron overload) and iron deficiency anemia. [1]

Methodology
Latex agglutination reactions occur as a result of antibody-coated latex beads aggregating if antigen is present in sufficient quantity. Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Under conditions of antibody excess, increasing amounts of antigen result in higher scatter. Turbidimeters measure the reduction of incident light due to reflection, absorption, or scatter.
In the Beckman Coulter procedure, the measurement of the decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay. The anti-ferritin reagent is a suspension of polystyrene latex particles, of uniform size, coated with polyclonal rabbit anti-ferritin antibody. When serum, containing ferritin, is mixed with the anti-ferritin reagent, an agglutination mixture occurs. This is measured spectrophotometrically on Beckman Coulter Chemistry Analyzers.

System Information
For AU400/400e/480, AU600/640/640e/680 and AU2700/5400/AU5800 Beckman Coulter Chemistry Analyzers.

Reagents
Final concentration of reactive ingredients
Glycine buffer (R1: pH 8.3, R2: pH 7.3) 170 mmol/L
Latex particles coated with rabbit anti-human ferritin
Preservative

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent and calibrator.
4. Dispose of all waste material in accordance with local guidelines.

Preparation of Reagents
R1 is ready for use and can be placed directly on board the instrument. The R2 latex solution should be mixed by inversion 5 – 10 times before placing on the instrument and at weekly intervals thereafter.

Storage and Stability
The reagents are stable, unopened, up to the stated expiry date when stored at 2 – 8°C. Once open, reagents stored on board the instrument are stable for 60 days.

Indications of Deterioration
Visible signs of microbial growth, gross turbidity, precipitate or change in color in the Ferritin reagent may indicate degradation and warrant discontinuation of use.

Specimen Collection and Preparation
Serum, Li-heparin plasma and EDTA plasma samples are the recommended specimens.
Strongly lipemic samples should be avoided.

Sample Storage and Stability
Stable in serum and plasma for 7 days when stored at 2 – 8°C and for 12 months when stored at -20°C.[2]

Interfering Substances
Results of studies[3] conducted show that the following substances interfere with this Ferritin procedure.
The criteria for no significant interference is recovery within 10% of the initial value or ≤ 5 ng/mL concentration change for samples containing high (170 – 223 ng/mL) or low (20 – 36 ng/mL) level Ferritin.
Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Hemolysis: No significant interference up to 500 mg/dL Hemolysate
Lipemia: No significant interference up to 400 mg/dL Intralipid*
RF: No significant interference up to 500 IU/mL RF

* Intralipid is a 20% IV fat emulsion used to emulate extremely turbid samples. Approximate triglyceride concentration is 1200 mg/dL.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[4] for a compilation of reported interferences with this test.

In very rare cases gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Ferritin

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.
Materials Provided
Ferritin Reagent

Materials Required But Not Provided
Serum Protein Multi-Calibrator (Cat No. ODR3021)

Stability of Final Reaction Mixture
The Beckman Coulter AU chemistry analyzers automatically compute every determination at the same time interval.

Calibration
The frequency of calibration for the Ferritin procedure is every 30 days. Ferritin values assigned to the calibrators are traceable to the 3rd International Standard for Ferritin, Recombinant NIBSC code: 94/572.

Recalibrate the assay every 30 days, or when the following occur:
1. Change in reagent lot or significant shift in control values,
2. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

Following calibration, the resulting curve should be visually reviewed, on the Beckman Coulter AU analyzer, for acceptability. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice

Quality Control
During operation of the Beckman Coulter AU chemistry analyzer at least two levels of an appropriate Immunology control material should be tested a minimum of once a day. In addition, these controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.
Please note that recovery of non-Beckman Coulter controls may vary with reagent lots of immunoassay products, due to the use of non-human materials in the controls.

Results
Automatically printed out for each sample in ng/mL at 37°C.

Limitations of the Procedure
This assay has been specifically designed to substantially reduce the risk of interference from HAMA or Heterophilic antibodies. However as with all immunoassays there is always a small risk from such interferences and therefore for diagnostic purposes the Ferritin results should always be assessed in conjunction with other available information e.g., patient's medical history, clinical impressions and results of other tests. [5,6,7]
To investigate samples which are believed to contain interference, a number of approaches can be adopted. Serial dilution may reveal incorrect recovery, PEG precipitation, pre-treatment with non immune serum or assay of the sample in an alternate assay system are all useful in identifying whether the sample contains an interferent. The results of such samples should be interpreted with extreme care.[7] Samples with extremely abnormal optical characteristics, especially turbidity, may produce atypical results. Such samples must be serially diluted and results compared to ensure that no such interference exists.

Dynamic Range
The Ferritin procedure is linear from 8 – 450 ng/mL with recovery within 10% or 3 ng/mL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN. Prozone or hook effect may occur with highly elevated Ferritin samples (>20,000 ng/mL).

Expected Values
A study[8,9] using the Ferritin assay on 125 healthy adult females and 154 males gave the following results:

Male: 16 – 243 ng/mL
Female: 10 – 158 ng/mL

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Specific Performance Characteristics
The following data was obtained using the Ferritin reagent on Beckman Coulter AU chemistry analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[10]
Estimates of precision, based on CLSI recommendations,[11] are consistent with typical performance. The repeatability precision is less than 8% CV and within laboratory precision is less than 11% CV. Assays of control material were carried out and data reduced following CLSI guidelines above.

The following data was obtained on an AU640 using 3 serum pools analysed over 20 days.

N= 80	Repeatability (Within Run)		Within Laboratory (Total)	
Mean, ng/mL	SD	CV%	SD	CV%
25	1.15	4.7	1.21	4.92
147	1.08	0.74	1.81	1.23
437	1.89	0.43	4.36	1.0

Method Comparison[10]

Patient samples were used to compare this Ferritin Reagent. The table below demonstrates representative performance on the AU chemistry analyzers.

Y Method	AU640
X Method	Method 2
Slope	1.06
Intercept (ng/mL)	4.3
Correlation Coeff. (r)	0.992
No. of Samples (n)	103
Range (ng/mL)	9.7 – 447.9

Analytical Sensitivity (Lower Detection Limit)

The lowest detectable level on an AU640 analyzer was calculated as 2.8 ng/mL

The lowest detectable level represents the lowest measurable level of Ferritin that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

Limit of Detection

The Limit of Detection (LOD) for the Ferritin reagent was determined to be less than 4.3 ng/mL The Limit of Detection (LOD) for the Ferritin reagent on the AU5800 analyzer was determined to be less then 4.6 ng/mL.This was determined according to CLSI protocol EP17-A.[12]

Limit of Quantitation

The Limit of Quantitation (LOQ) for the Ferritin reagent was determined to be less than 7.8 ng/mL. This was determined according to CLSI protocol EP17-A[12] and represents the lowest concentration of Ferritin that can be measured with a total imprecision of 20%.

References

1. Henry JB [ed] Clinical diagnosis and management by laboratory methods, 19th ed. Philadelphia: WB Saunders Company, 1996.
2. WHO. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1 rev.2, 2002:31.
3. CLSI, Interference testing in clinical chemistry, EP7-A2, 2005.
4. Young DS. Effects of drugs on clinical laboratory tests, AACC, 5th ed. AACC Press, 2000.
5. Selby C. Interference in immunoassay. Ann Clin Biochem 1999; 36: 704-721.
6. Ismail AA. On the interpretation of affirmative follow-up tests in immunoassays: what must not be done? Ann Clin Biochem 2006; 43(4): 249-251.
7. CLSI, Immunoassay interference by endogenous antibodies; proposed guideline, I/LA30-P, 2007.
8. Data is on file at Beckman Coulter Inc.
9. CLSI, Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline, C28-A3, 2008.
10. Data is on file for specific AU chemistry analyzers.
11. CLSI, Evaluation of precision performance of quantitative measurement methods, EP5-A2, 2004.
12. CLSI, Protocol for determination of limit of detection and limit of quantitation; Approved guideline, EP17-A, 2004.

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