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| **Ferritin** |
| **Purpose** | The LOCI FERR method is an *in vitro* diagnostic test for the quantitative measurement of ferritin in human serum and plasma on the Dimension Vista® System. |
| **Policy Statements** | This procedure applies to all personnel who run the Dimension Vista 500. |
| **Principle** | The LOCI FERR method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI® technology. The LOCI® reagents include two synthetic bead reagents and a biotinylated anti-ferritin monoclonal antibody fragment. The first bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. The second bead reagent (Chemibeads) is coated with a second anti-ferritin monoclonal antibody and contains chemiluminescent dye. Sample is incubated with biotinylated antibody and Chemibeads to form a particle/ferritin/biotinylated antibody sandwich. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is a direct function of the ferritin concentration in the sample.Notice: This assay is sensitive to higher than normal levels of supplemented Biotin. Results may be falsely decreased, depending on the level of Biotin in circulation.  |
| **Clinical Significance** | High concentrations of ferritin are found in liver cells and in erythrocyte recycling centers (RE cells) of the liver, spleen and bone marrow. In these tissues, ferritin serves as the body's principal storehouse for surplus iron, protecting against the toxic effects of excess and maintaining a readily mobilized reserve for erythropoiesis.Ferritin is also present in human plasma, where its concentration is normally a satisfactory index of body iron stores.Concentrations below 10 or 15 ng/mL are typical of uncomplicated iron deficiency anemia. For iron overload, values over 300 or 400 ng/mL are the rule, with levels in the 1 000 – 5 000 ng/mL range common in full-blown cases of hemochromatosis. It has important roles to play in the diagnosis of iron deficiency and excess, and in the management of conditions and treatments posing a threat to iron balance. It has proved a valuable aid in discriminating iron deficiency anemia from anemias due to other causes and in exposing the disappearance of iron reserves before the onset of anemia. Iron deficiency anemia (IDA) is common among menstruating and reproductively active females, children, older adults, and vegetarians. A low ferritin level is an early indicator of IDA; occurring before serum iron is decreased and morphological abnormalities appear in red blood cells. Normal ferritin levels cannot be used to exclude IDA if a hepatic, malignant or inflammatory condition exists in the patient (anemia of chronic disease, ACD). Patients with ACD may show normal or slightly increased ferritin levels caused by the acute phase response associated with chronic inflammation, which overrides the decrease in ferritin associated with IDA. Although iron depletion appears to be the only condition associated with reductions in the serum ferritin level, increases are observed not only in the presence of increased iron stores but also in several other situations, including liver disorders, inflammatory conditions, leukemia, Hodgkin's disease and certain other malignancies. Increased levels may reflect the escape of ferritin from damaged liver cells, impaired clearance of ferritin from the plasma, synthesis of ferritin by tumor cells, or an expansion of the iron storage compartment induced by ineffective erythropoiesis. Inflammation tends to raise the ferritin level while lowering the serum iron concentration by stimulating increased ferritin production in RE cells. |
| **Instrument** | PRIMARY METHOD: Siemens Dimension Vista 500SECONDARY (BACKUP) METHOD: Siemens Dimension Vista 500 on opposite campus |
| **Sunquest Test Code** | FERI Ferritin ng/mL |
| **Sample** | **Preferred Sample**:Lithium heparin plasma**Also Acceptable**: Serum (red, marble or gold). Refer to the Specimen Collection Manual for collection of specimens.**Minimum Volume:**200μL preferred, 100μL minimum, 2 μL actual test volume**Stability:**7 days/2-8°C, or 6 months at -20°C.Serum samples are stable for up to 24 hours at room temperature on the clot.Freeze samples only once and mix thoroughly after thawing.**Specimen Rejection:*** Samples and controls stabilized with sodium azide cannot be used
* Unlabeled
* Avoid using grossly hemolyzed samples.

**Preparation:**1. Complete clot formation should take place before centrifugation to prevent the appearance of fibrin in serum samples. Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
2. Serum or plasma should be physically separated from cells with a maximum limit of 2 hours from the time of collection. Specimens should be free of particulate matter.
3. Transfer serum or plasma to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
4. Very lipemic or frozen samples that become turbid after thawing must be clarified by centrifugation before testing.
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| **Reagents** |

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| FERR Flex® reagent cartridge All reagents are liquid and ready to use. | K6440 | **Store at:** 2 - 8 °C.**Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days.**Open well stability:** 7 days for wells 1 - 12. |
| LOCI 4 CAL | KC640A  | **Store at:** -25 to -15 °C.**Unopened:** Refer to carton for expiration date.**Preparation:** Thaw and equilibrate at 22 – 28 C for 30 minutes. Mix by inverting 10 times.**On-board:** Once the vial stopper is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista® System**Opened:** Once the cap is removed, the assigned values are stable for 30 days when recapped immediately and stored at 2 - 8 °C. Do not use this vial on board. |

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| **Risk and Safety** | Irritant. Contains a mixture of 5-chloro-2-methyl-2H-isothiazol -3-one and 2‑methyl‑2H‑isothiazol-3-one (3:1).* May cause sensitization by skin contact.
* Avoid contact with skin.
* Wear suitable gloves.
* Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics
* Used LOCI® reaction vessels contain human body fluids; handle with appropriate care to avoid skin contact or ingestion. Reaction vessels are designed for single use only.
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| **Calibration** |

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| Assay Range: | 0.5 – 2000 ng/mL |
| Calibration Material: | LOCI 4 CAL, PN. KC640A |
| Calibration Scheme | 5 levels, n=3Load levels A through E on the Vista prior to calibration |
| Typical Calibration Levels | Level 1 (Calibrator A): 0 ng/mLLevel 2 (Calibrator B): 25 ng/mLLevel 3 (Calibrator C): 210 ng/mLLevel 4 (Calibrator D): 1050 ng/mLLevel 5 (Calibrator E): 2000 ng/mL |
| Calibration Frequency: | * Every 30 days for any one lot
* For each new lot of Flex® reagent cartridges
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
* When required by government regulations
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Consult the Vista Calibration Procedure or your Vista iGuide for calibration instructions. |
| **Analytical Measuring Range (AMR)** | Cal Verification and AMR verification requirements are met by the Ferritin method calibration using 5 calibrators that span the full measuring range. |
| **Quality Control** | **Product**: Biorad Immunoassay Plus Control in Vista Vials, 2 levels. **Preparation**: Allow the frozen control to stand at room temperature (18 to 25°C) until it is completely thawed. Gently swirl the contents of the vial and immediately load the vial onto the instrument.**Frequency:** Two levels once each day of patient testing.**Stability:** This product will be stable until the expiration date when stored unopened at -20°C to -50°C.Thawed and stored at 2 to 8°C or on board the Siemens Dimension Vista, all analytes will be stable for 4 days. Once thawed, do not refreeze the control; discard the remaining material.**Sunquest Control Names:** Level 1 = C-IMV1Level 3 = C-IMV3.**Acceptable Ranges:** Ranges are current in Sunquest and the instrument. Refer to the [Quality Control in Chemistry](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Quality/201755.pdf) Procedure for QC exception codes. |
| **Interferences** | Interfering substances:This assay is sensitive to increased levels of Biotin due to supplementation. At a level of 20 mg/dL Ferritin in the blood with 1200 ng/mL of Biotin, the values may be decreased by -16.8%. No interference was found for:* Commonly used drugs.
* HIL:
	+ Hemoglobin (free) up to 500 g/dL
	+ Bilirubin (conjugated) up to 60 mg/dL
	+ Bilirubin (unconjugated) up to 20 mg/dL
	+ Lipemia (Intralipid®) up to 3000 mg/dL

Non-Interfering Substances:Refer to the product insert for a list of substances that were shown to have no measurable effect on the Ferritin result at typical concentrations.**Hook Effect**One-step sandwich immunoassays are potentially susceptible to a high dose “hook effect,” where an excess of antigen prevents simultaneous binding of the capture and detection antibodies to a single analyte molecule. The FERR method shows no hook effect up to at least 250,000 ng/mL |
| **Reference Range** |

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| Age | Males ng/mL | Females ng/mL |
| <1 month | 25-200 | 25-200 |
| 1-2 months | 200-600 | 200-600 |
| 2-5 months | 50-200 | 50-200 |
| 6 months-15 yrs | 7-142 | 7-142 |
| >16 yrs | 22-322 | 10-291 |

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| **Critical Values** | None specified. |
| **Limitations** | Performance of this assay has not been established by Siemens with pediatric specimens.The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in ferritin results. Refer to your Dimension Vista® Operator’s Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed according to your laboratory’s procedure manual and not reported.Patient samples may contain heterophilic antibodies that could react in immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution. |
| Dilutions |

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| Surplus Rack | Samples with results >2000 ng/mL are repeated on a higher dilution (1:20). |
| Limited Rack | Samples with results >560 mg/dL should be repeated as an Add-On Test with a Special Dilution of 1:20 |
| Maximum Dilution | 1:20 performed by Vista |

Refer to the Vista iGuide for instructions on ordering Special Dilutions. |
| **Result Reporting** | * Results between 0.5-2000.0 ng/mL without error messages are released
* All results will have appended comment “Supplemented Biotin may falsely decrease this assay. Repeat the testing 24 hours after last biotin dose.”
* Results below 0.5 ng/mL: report as < 1 ng/mL instead of the numerical value.
* Results >2000 ng/mL are reported following a maximum dilution of 1:20
* Results with “assay range” appended following a maximum dilution of 1:20 are reported as >40,000 ng/mL
* Gross Hemolysis: Append the comment “**HP**” (Hemolysis Present, May Affect Results) to the Ferritin result
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in numbered specimen rack by accession number. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. LOCI Ferritin Flex® reagent cartridge for Dimension Vista® System, Siemens Healthcare Diagnostics Inc., Newark, DE 19714, US. 2015-03-25 E PN 781440.001 - US.
2. Siemens Dimension Vista LOCI 4 CAL Product Insert, KC640A, 3-13-2014
3. Biorad Immunoassay Plus Control Product Insert
4. Burtis, CA, Ashwood, ER, Bruns, DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th Edition, Philadelphia: W.B. Saunders, 2006.
5. Jacobs and DeMott Laboratory Test handbook, 5th Edition, Lexi-comp Inc. 2001
6. Siemens Healthcare Diagnostics Customer Bulletin, 11313747, Rev. A, 2017
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | C. Bryant/L.Lichty | January 2005 | Replaces Ferritin on ACS180: SE |
|  | L.Lichty  | September 25, 2006 | Ferritin on Immulite 2000 |
|  | L. Lichty | May 21, 2009 | Corrected reportable range, added hemolysis comment |
|  | L. Lichty | July 1, 2011 | Updated product insert, renumbered from CH 1.03 |
|  | L. Lichty | March 8, 2013 | Clarify maximum dilution reporting |
|  | L. Lichty | July 22, 2014 | Replaces Ferritin on Immulite 2000 |
|  | Erin Bartos | June 26, 2017 | Biennial Review, updated sample type and IFU  |
|  | Erin Bartos | January 8, 2018 | Biotin interfering substance information added |
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