| **Insulin-like Growth Factor Binding Protein 3 (IGFBP-3)** |
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| **Purpose** | This procedure provides instructions for performing INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN- 3 (IGFBP-3) on the IDS iSYS. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the IDS iSYS. |
| **Principle** | The IGFBP-3 assay is based on chemiluminescence technology. 6 μL of patient sample or calibrators are diluted in a diluent and a portion of this is incubated with a biotinylated anti-IGFBP-3 monoclonal antibody and an acridinium labeled anti-IGFBP-3 antibody for a period of time. Streptavidin coated magnetic particles are then added for a further incubation. The magnetic particles are captured using a magnet and a wash step performed to remove any unbound analyte. Trigger reagents are added; the resulting light emitted by the acridinium label is directly proportional to the concentration of IGFBP-3 in the original sample. |
| **Clinical Significance** | The IGF system is well characterized and plays a critical role in the growth and differentiation of normal and malignant cells. The components of the IGF-I system include Growth Hormone, IGF-I and II, type I and II receptors, IGF binding proteins and proteases. Insulin-like growth factor binding proteins were first identified for their high affinity interactions with IGF-I and IGF-II. A consensus was reached on the nomenclature for IGF-binding proteins (IGFBPs) of which there are 6 members (IGFBP-1-6). IGFBP-3 is an abundant IGFBP species in circulation and binds 75% to 90% of circulating IGF-I in a 150kDa ternary complex consisting of IGFBP-3, IGF-I and acid-labile subunit (ALS). IGFBP-3 has a molecular weight of 28.7 kDa, comprises 264 amino acids, and acts to modulate the activity of IGF I and II and to increase their half life. It has been postulated that IGFBP-3 is regulated by GH and originates in the liver as low levels were observed in patients with impaired hepatic function. IGFBP-3 values are used in the investigation of growth hormone deficiency (pediatric and adult), acromegaly, and hypopituitarism and to monitor rhGH therapy. GH deficient patients have subnormal IGFBP-3 levels and determination of the IGFBP-3 concentration is sufficient for the diagnosis of GH deficiency with high confidence. |
| **Instrument** | **PRIMARY METHOD: IDS iSYS****Backup Method: Mayo Medical Laboratories (IGFB3)** |
| **Sunquest Test Code** | IGFB |
| **Specimen** | **Sample type Preferred: Red No Gel**  Also acceptable: Green No Gel **Preferred Draw Volume**: 1.8 mL (Minimum 0.6 mL)**Preferred Sample Volume:** 0.6 mL (Minimum 0.2 mL) Note: Minimum volume does not allow for repeat analysis.**Stability When Separated Within 1 Hour of Draw:** 8 hours at Room Temperature, 24 hours at 2-8°C, 4 weeks -20°C or colder. Avoid repeated freeze-thaw of samples.**Shipping Temperature:** Draw sites other than Minneapolis should ship samples frozen.**Rejection criteria:** Unlabeled specimens, incorrect sample type, samples from patients currently receiving biotin supplementation**Preparation:** 1. Serum specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. Plasma specimens can be centrifuged immediately.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of one hour from the time of collection.
3. Lipemic samples should be ultrafuged.
4. Specimens should be free of particulate matter.
5. Frozen specimens must be completed thawed and fully mixed by end to end inversion several times to ensure homogeneity prior to testing.
6. Transfer serum or plasma to a properly labeled sendout tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
7. Sample volumes less than 0.5 mL must be transferred to an IDS sample cup by the testing staff. This sample cup must be properly labeled at all times, as described in step 5.
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| **Reagents** |

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| ***Product Description*** | ***Product*** | ***Stability*** |
| IDS iSYS IGFBP-3 Reagent  | IS-4400 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer expiration date**Open:** 28 Days**On-board:** 14 Days |
| IDS iSYS IGFBP-3 Calibrator (Included in Reagent Kit) | IS-4400 | **Store at:**  -20°C**Unopened**: Manufacturer expiration date**Opened**: 4 weeks at -20°C, stored in original vials**On Board:** 3 hours**Instructions:** Reconstitute with 1.0 mL of DI water. Replace. Let sit for 10 minutes, swirling occasionally by hand. If frozen in aliquots, calibrator must be put in the freezer within 15 minutes of reconstitution. Freeze only once. When thawed, must be at room temperature prior to testing and must be tested within 30 minutes of thaw. |
| IDs iSYS IGFBP-3 Controls | IS-4430 | **Store at:** 2 – 8 °C**Instructions:** Add exactly 1.0 mL DI water. Replace the stopper. Leave for 10 minutes to reconstitute with occasional mixing by hand before use. Avoid the formation of foam. Following reconstitution and within 15 minutes, IGFBP-3 Controls should be aliqoted for single-use only in 200uL portions, frozen at -20°C. It is advised that IGFBP-3 controls not be used directly after reconstitution. Instead, all control aliquots should undergo exactly 1 freeze-thaw cycle before use. Thawed controls should be brought to room temperature and mixed well prior to use. They should be loaded on the System within 30 minutes of thaw. Do not re-freeze QC once thawed.**Open Stability:** Aliquots are stablefrozen at -20°C for 28 days. When thawed and onboard the analyzer, they are stable for 3 hours. |
| IDS-iSYS Cuvettes  | IS-CC100 |  |
| IDS-iSYS System Liquid  | IS-CS100 | **Store at:** 15-25°C **Open Stability:** Managed by System Software |
| IDS-iSYS Wash Solution  | IS-CW100 | **Store at:** 15-25°C **Open Stability:** Managed by System Software |
| IDS-iSYS Triggers Set  | IS-CT100 | **Store at:** 15-25°C **Open Stability:** Managed by System Software |
| IDS-iSYS Cartridge Check System  | IS-6010 | **Store at:** 2-8°C**Unopened Stability:** Until Manufacturer Expiration Date**Open Stability:** 9 weeks**Open and loaded on the Analyzer:** 8 weeks |
| D-SORB solution  | IS-DS200 | **Store at:** 15-25°C**Open Stability:** Managed by System Software |
| IDS iSYS IGFBP-3 Calibration Verifier | IS-4435 | **Store at:** 2-8°C**Open Stability**: 2.5 hours onboard the analyzer  |

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| **Risk and Safety:** | Some reagents in this kit contain sodium azide <0.1 % (w/w) which may react with lead, copper or brass plumbing to form highly explosive metal azides. Dispose of in appropriate hazardous waste container:* **Trigger A**: Recap and dispose of in Hazardous Acid waste container (acid flex waste.)
* **Trigger B**: Recap and dispose of in Hazardous Basic/Caustic waste container (base flex waste.)
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| Calibration/ Calibration Verification/AMR |

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| Analytical Measuring Range: | 0.08 mg/L- 10.00 mg/L |
| Reference Material: | IGFBP-3 Calibrator (included in IGFBP-3 Reagent, IS-4400) |
| Suggested Calibration Levels | A – 0.0 mIU/mLB – 10 mIU/mL |
| Calibration Scheme: | n=2 |
| Calibration Frequency: | * For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
* Every 14 days
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| Calibration Verification/AMR | Verification of AMR is accomplished with the IGFBP-3 Calibration Verifier materials and entering the results into EP Evaluator. Results are reviewed by the Technical Specialist for acceptability.* Cal Verification and AMR verification are performed at least once every six (6) months.
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| **Quality Control** | **IDS iSYS IGFBP-3 Controls Level 1, 2, 3** **Frequency:** 3 Levels, once per shift of use (day and evening shift)**Stability:** Unopened: Until manufacturer expiration date when stored at 2 – 8 °C. Opened and reconstituted: 28 days at -20°C**Preparation**: Add exactly 1.0 mL DI water. Replace the stopper. Leave for 10 minutes to reconstitute with occasional mixing by hand before use. Avoid the formation of foam. Following reconstitution and within 15 minutes, IGFBP-3 Controls should be aliqoted for single-use only in 200uL portions, frozen at -20°C. It is advised that IGFBP-3 controls not be used directly after reconstitution. Instead, all control aliquots should undergo exactly 1 freeze-thaw cycle before use. Thawed controls should be brought to room temperature and mixed well prior to use. They should be loaded on the System within 30 minutes of thaw. Do not re-freeze QC once thawed.**Sunquest Control names:** Level 1 =BP1 , Level 2 = C-BP2, Level 3= C-BP3**Acceptable ranges:** * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
* Do not release patient results until the cause of deviation has been identified and corrected
* When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
* When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 20 times, and calculate a new range using the method mean ± 2 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
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| **Interferences** | * Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
* Biotin interferes with this assay and is not appropriate for patients receiving biotin supplementation. Allow at least 24 hours post-supplementation of biotin-containing medication before testing.
* The hook effect was tested using concentrations of IGFBP-3 up to 100000 ng/mL. No hook effect was observed.
* The IDS iSYS analyzer does not contain a level-sensing mechanism. Therefore, every sample must be checked for bubbles or fibrin prior to loading the sample on the analyzer. Any questionable results must be checked by repeat after rechecking the sample for the presence of these testing interferences.

**Interferences**:Lipid > 3000 mg/dLBilirubin >200 mg/dLHemoglobin >500 mg/dlBiotin >300 nmol/LRed Blood Cells >0.4% |
| **Reference Intervals** |

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| **IGFBP-3 Reference Intervals** |
|   | Male | Female |
| 0-11 mo | 1.11-3.18 | 1.05-3.27 |
| 1 year | 1.29-3.63 | 1.22-3.72 |
| 2 year | 1.47-4.07 | 1.39-4.15 |
| 3y | 1.64-4.49 | 1.55-4.56 |
| 4y | 1.80-4.88 | 1.71-4.93 |
| 5y | 1.94-5.19 | 1.85-5.24 |
| 6y | 2.04-5.38 | 1.95-5.40 |
| 7y | 2.10-5.47 | 2.02-5.52 |
| 8y | 2.15-5.55 | 2.10-5.63 |
| 9y | 2.22-5.66 | 2.18-5.76 |
| 10y | 2.30-5.80 | 2.27-5.91 |
| 11y | 2.39-5.96 | 2.36-6.06 |
| 12y | 2.46-6.09 | 2.44-6.18 |
| 13y | 2.53-6.20 | 2.52-6.29 |
| 14y | 2.58-6.27 | 2.58-6.37 |
| 15y | 2.61-6.31 | 2.64-6.43 |
| 16y | 2.64-6.32 | 2.68-6.47 |
| 17y | 2.66-6.32 | 2.72-6.50 |
| 18y | 2.68-6.33 | 2.75-6.51 |
| 19y | 2.70-6.34 | 2.78-6.53 |
| 20y | 2.72-6.36 | 2.81-6.55 |
| 21-25y | 2.75-6.36 | 2.86-6.56 |
| 26-30y | 2.68-6.13 | 2.75-6.22 |
| 31-35y | 2.61-5.98 | 2.57-5.80 |
| 36-40y | 2.57-5.98 | 2.50-5.71 |
| 41-45y | 2.52-6.02 | 2.41-5.61 |
| 46-50y | 2.37-5.89 | 2.34-5.61 |
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| Tanner Stages | Male | Female |
| I | 2.71-5.26 | 2.80-5.24 |
| II | 3.53-5.75 | 3.05-5.60 |
| III | 3.73-6.39 | 3.93-6.01 |
| IV | 3.37-6.20 | 3.80-6.36 |
| V | 3.87-6.65 | 3.88-6.49 |

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| **Critical Values** | None specified |
| **Limitations** | * The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages.
* This assay is affected by biotin interference. See interfering substances for more information.
* As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient’s clinical presentation and other information available to the physician.
* Partially thawed or improperly mixed samples will give erroneous results.
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| **Dilutions** | Do not dilute. |
| **Result Reporting** | 1. Results <0.08 mg/L should be reported as <0.08 as opposed to the numerical value.
2. Results >10.0 mg/L should be reported as >10.00 rather than the numerical value. **Do not dilute.**
3. In Sunquest, utilize the OEM function to review all results under the method code ISYS.
4. Results will show reference interval flags based on the physical age of the child. Tanner Stage Reference Intervals will append to each result based on the patient sex.
5. Any result below 0.2 mg/L must be repeated after physically checking the sample for bubbles or fibrin.
6. The sample of any questionable result must be checked and the test repeated prior to resulting.
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| **Specimen Storage** | After testing completion, samples are stored frozen for 2 weeks in the Special Chemistry Freezer.  |
| **References** | 1. IDS-iSYS IGFBP-3 Package Insert, Immunodiagnostic Systems Limited, Gaithersburg, MD 20878. Revised February 2017.
2. IDS-iSYS Cartridge Check System (CCS) Package Insert. IFU Version: V05. Effective Date November 18, 2016.
3. IDS iSYS IGFBP-3 Control Set Package Insert, Immunodiagnostic Systems Limited, Gaithersburg, MD 20878. IS-4430 v06 Updated September 07, 2017.
4. IDS iSYS IGFBP-3 Calibration Verifier Package Insert, Immunodiagnostic Systems Limited, Gaithersburg, MD 20878. IS-4435PL V04, January 21, 2015.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/Erin Bartos | 6/19/2018 | New Procedure |
| 2 | Erin Bartos | 6/25/18 | Updated Backup Method to Mayo Medical Laboratories |
| 3 | Erin Bartos | 7/9/2018 | Clarification regarding thawed samples, repeat testing, and test interferences by bubbles and fibrin.  |

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