| **Human Growth Hormone (hGH)** |
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| **Purpose** | This procedure provides instructions for performing HUMAN GROWTH HORMONE on the IDS iSYS. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing IDS iSYS analyzer. |
| **Principle** | The assay is based on chemiluminescence technology. 50 μL of patient sample or calibrators are incubated with a biotinylated monoclonal anti-hGH antibody and streptavidin labeled magnetic particles. The magnetic particles are “captured” using a magnet and a wash step performed. An acridinium labeled anti-hGH monoclonal is added and following a further incubation step a second wash step is performed. Trigger reagents are added; the resulting light emitted by the acridinium label is directly proportional to the concentration of hGH in the original sample. |
| **Clinical Significance** | Growth hormone (hGH) is a polypeptide hormone secreted from the acidophil cells of the anterior pituitary gland. Secretion is episodic and is associated with exercise, the onset of deep sleep or post-prandially in response to falling glucose levels. Synthesis and release are under the control of hypothalamic releasing peptides and inhibitory peptides such as somatostatin. More recently, a gastric peptide, Ghrelin, has been shown to also stimulate hGH secretion. In contrast, the mediator of many hGH actions in the periphery, insulin-like growth-factor I (IGF-I) exerts an inhibitory effect through negative feedback mechanisms. HGH in circulation consists of several molecular isoforms, with 22,000 Dalton hGH being the most abundant, followed by a 20,000 Dalton hGH variant produced by alternative splicing. Approximately 50% of circulating hGH is bound to a high affinity binding protein. HGH is physiologically important in two main areas. First, it has an integral role in skeletal growth which is well demonstrated in either excess or deficiency in childhood. The action of hGH in part is mediated through IGF-I as well as promoting protein synthesis and the uptake of amino acids into cells. Secondly, hGH influences intermediary metabolism by stimulating lipolysis and is antagonistic to the insulin-mediated uptake of glucose. HGH secretion is stimulated by hypoglycemia and suppressed by hyperglycemia. In childhood, symptoms of hGH deficiency are retarded growth and dwarfism. Etiology is often unknown and an absolute or relative deficiency usually becomes apparent at about 2 years of age. Diagnosis can be confirmed by demonstrating low serum hGH which does not respond to stimulation tests. HGH deficiency is a major cause of severe short stature and diagnosis at an early stage is essential for successful therapy. Hyposecretion in adults usually becomes apparent during the laboratory investigation of hypopituitarism. Hypersecretion, commonly due to adenoma of the acidophil cells, is characterized by two conditions depending on whether it becomes apparent before or after fusion of the bony epiphyses. In childhood, excess hGH is characterized by gigantism. Heights of 8 feet (2.4 meters) may be achieved and may also be associated with hypogonadism. In adults acromegaly results in a condition characterized by progressive thickening of bone and soft tissue. Diagnosis is usually confirmed by dynamic function testing which demonstrates a raised serum hGH level which does not fall in response to an oral glucose load. In conditions where there are nutritional disturbances such as anorexia, starvation, renal failure and hepatic cirrhosis, increased basal hGH levels may be found. Recombinant hGH is available for treatment of hGH deficiency in both children and adults. HGH excess is treated by surgery, irradiation therapy or somatostatin analogues. More recently, a hGH receptor antagonist has been developed, which shares structural homology to hGH and competes with hGH for binding to the hGH receptor. |
| **Instrument** | **PRIMARY METHOD: IDS iSYS****BACKUP METHOD: Esoterix Laboratory** |
| **Sunquest Test Code** | HGH |
| **Specimen** | **Preferred Sample Type**: Red No Gel. Also acceptable: SST**Preferred Draw Volume**: 2.1 mL blood (1.0 mL minimum)**Preferred Sample Volume**: 0.7 mL serum (0.3 mL minimum)Note: minimum volume does not allow for repeat analysis.**Stability When Separated Within 1 Hour of Draw**: 12 hours at room temperature/2-8°C, 4 weeks at -20°C or colder. **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.
2. Serum 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 samples must be completely 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 volume less than 500uL should be transferred to an iSYS sample cup by the tech performing the testing. Sample cups 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 hGH Reagent  | IS-3700 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer expiration date.**Open:** 28 Days**On-board:** 11 Days |
| IDS iSYS hGH Calibrator (Included in Reagent box) | IS-3700 | **Store at:**  -20°C**Unopened**: Manufacturer expiration date.**Opened**: 7 weeks at -20°C**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. |
| Bio-Rad Lyphochek Immunoassay Plus QC, LYIP1, LYIP2, LYIP3 | BR 371,372, 373 | **Store at:** 2-8°C **Instructions:** Reconstitute with 5mL of DI water let vials sit for 15 minutes and gently swirl. **Open Stability:** 7 days at 2-8°C. |
| IDS-iSYS Cuvettes  | IS-CC100 | **Store at:** 15-25°C |
| System Liquid  | IS-CS100 | **Store at:** 15-25°C**Open Stability:** Managed by System Software |
| Wash Solution  | IS-CW100 | **Store at:** 15-25°C**Open Stability:** Managed by System Software |
| IDS-iSYS Triggers Set (A and B)  | IS-CT100 | **Store at:** 2-8°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 |
| hGH Calibration Verifier Set | IS-3735 | **Store at:** 2-8°C, stable until manufacturer expiration date unopened**Open Stability:** 2.5 hours onboard the analyzer |

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| **Risk and Safety:** | Contains sodium azide. Avoid contact with skin and eye. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. Dispose of in appropriate Hazardous Waste Container. |
| Calibration/ Verification/AMR |

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| Analytical Measuring Range: | 0.05-100.00 ng/mL  |
| Reference Material: | hGH Calibrators are included in the Reagent (IS-3700.)hGH Cal Verifier (IS-3735) |
| Suggested Calibrator Levels | A – 0.193 ng/mLB – 19.503 ng/mL |
| Calibration Scheme: | n=2 |
| Calibration Frequency: | * Every 7 Days
* For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| Calibration Verification/AMR | Verification of AMR is accomplished with the hGH Cal 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** | **Bio-Rad Lyphochek Immunoassay Plus 1, 2, 3****Frequency:** Three levels each shift of use (day and evening.)**Stability:** 7 Days at 2-8°C.**Preparation**: Reconstitute with exactly 5.0 mL of DI water. Allow to stand for 15 minutes at room temperature, swirling occasionally to ensure homogeneity. Stopper immediately after use and return immediately to refrigerated storage.**Sunquest Control names:** Level 1 = C-LYIP1, Level 2 = C-LYIP2, Level 3= C-LYIP3Instrument Code: ISYS**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 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 pre-analytical test interferences.

**Interfering Substances:**Lipids >3000 mg/dLBilirubin >200 mg/dLHemoglobin >500 mg/dLBiotin >300 nmol/L Growth Hormone Binding Protein >140 ng/mL |
| **Reference Range** | **Age**  **Reference Interval**0-1 day 5-53 ng/mL1-13 days 5-27 ng/mL14 days to 12 months 2-10 ng/mL>1 year 0-6 ng/mLResponse Testing, Children and Adults: Following provocative stimuli, a normal response is a rise to 10 ng/mL or greater. Intermediate values between 7 and 10 ng/mL may indicate a partial deficiency and require further evaluation. |
| **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 <0.05 ng/mL should be reported as <0.05 as opposed to the numerical value.
2. Results >100.00 ng/mL should be reported as >100.00 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. The samples of any questionable results must be physically checked for the presence of bubbles or fibrin prior to repeat testing.
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| **Specimen Storage** | After testing completion, samples are moved within 8 hours to the Special Chemistry Freezer and stored frozen for 2 weeks.  |
| **References** | 1. Human Growth Hormone Reagent Package Insert, Immunodiganostic Systems, Gaithersburg, MD 20878. IS-3700PL v04 Revised January 31, 2017.
2. Lyphochek Immunoassay Plus Control Package Insert, Bio-Rad Laboratories, Irvine, California. Updated January 2017.
3. Human Growth Hormone Cal Verifiers Package Insert IFU Version: 05 22 December 2014
4. IDS-iSYS User Manual - Revision M1 IA, Software version V 14.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/Erin Bartos/Stephen Gripentrog | 6/12/2018 | New Procedure: HGH transitioned from Immulite to iSYS. |
| 2 | Erin Bartos | 7/9/2018 | Clarified questionable samples due to fibrin, bubbles, or those that are thawed and improperly mixed.  |
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