



Laboratory Service Manual

Lab Dept: Anatomic Pathology

Test Name: GAA FULL GENE SEQUENCING

General Information

Lab Order Codes: GAAS

Synonyms: Pompe, Glycogen Storage Disease II; Acid a-glucosidase (GAA) gene sequencing for Pompe Disease; GAA DNA Full Sequencing Assay; GAA FULL;GAA; GSD II

CPT Codes: 83909 –Molecular diagnostics; separation and identification by high resolution technique (eg, capillary electrophoresis), each nucleic acid preparation
83891 – Molecular diagnostics; isolation or extraction of highly purified nucleic acid, each nucleic acid type (ie, DNA or RNA)
83894 – Molecular diagnostics; separation by gel electrophoresis (eg, agarose, polyacrylamide), each nucleic acid preparation
83892 – Molecular diagnostics; enzymatic digestion, each enzyme treatment
83898 x16 – Molecular diagnostics; amplification, target, each nucleic acid sequence
83904 x16 – Molecular diagnostics; mutation identification by sequencing, single segment, each segment

Test Includes: Testing includes coverage of the common adult-onset mutation in intron 1 (c.-32-13T>G). Patient sequences are compared to the reference DNA sequence (GenBank Accession: NT_024871.11).

Logistics

Test Indications: Patients with clinical symptoms consist with Pompe disease or deficient GAA enzyme activity as well as individuals with a family history of Pompe disease should be tested.

Lab Testing Sections: Anatomic Pathology - Sendouts

Referred to: Duke University Molecular Diagnostics Laboratory

Phone Numbers:

Minneapolis: 612-813-6280

Saint Paul: 651-220-6550

Test Availability: Restricted Draw Time: Draw Monday thru Thursday before 2pm



Laboratory Service Manual

Turnaround Time:	Results within 14 – 30 days.
Special Instructions:	Restricted draw times. See Test Availability . Requests must include request form with patient's name, date and time of collection, collector's initials, indications for study, ethnicity of patient and completed informed consent form. All forms can be found at Duke Molecular: Medical Genetic Requisition Form

Specimen

Specimen Type:	Whole blood
Container:	Lavender (EDTA) top tube
Draw Volume:	5 mL (Minimum: 3 mL) whole blood
Processed Volume:	Same as Draw Volume
Collection:	Routine venipuncture
Special Processing:	Lab Staff: Do Not centrifuge. Forward unprocessed peripheral blood promptly to the laboratory at ambient temperatures. Storage greater than 24 hours should be refrigerated.
Patient Preparation:	Due to the unique nature of genetic testing, patients offered this test should receive pre-test and post-test genetic counseling. Counseling should help the patient understand the strengths and limitations of DNA testing and the medical implications for the patient as well as for other family members. Patients are also required to give consent for testing.
Sample Rejection:	Sample not received by reference lab within 48 hours of draw; mislabeled or unlabeled specimens; frozen specimens; specimens other than EDTA whole blood

Interpretive

Reference Range:	An interpretive report will be provided
Critical Values:	N/A



Laboratory Service Manual

Limitations:

The sensitivity of DNA sequencing is 99% for the detection of nucleotide base changes and small deletions and insertions in the regions analyzed. Only the coding regions of the GAA gene and immediate flanking intronic sequences are examined. Changes in the promoter region, farther into the introns, or in other non-coding regions of the gene, would not be detected. Mutations in genes other than GAA would not be identified. Large deletions, duplications, multiple exon insertions, sequence alterations adversely affected primer binding, and complete deletion of one allele may not be identified using these methods. Using a similar sequencing strategy, Hermans et al. identified 53 of the 58 mutant alleles in 29 unrelated patients with either infantile or adult-onset Pompe disease, indicating that 91% of disease-causing alleles can be detected by full gene sequencing. Hermans et al. (2004) Human Mutation 23:47-56.

Methodology:

The protein coding sequences and flanking intronic sequences (minimum of 20 base pairs) of the GAA gene are amplified from purified genomic DNA by PCR. The primers used in these PCR reactions contain M13 universal primer "tails" at their 5' ends, and have 3' ends that are homologous to their genomic target sequence. The resulting PCR products are treated with an exonuclease/ phosphatase mixture (ExoSAP-IT) to remove excess PCR primers and nucleotides. These purified DNA amplicons are then sequenced using universal M13 forward and reverse primers (M13 Forward/-20 and M13 Reverse/-27) and the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem). These products are purified with the Big Dye XTerminator Purification Kit and resolved using the ABI 3130xl Genetic Analyzer. Data is analyzed using the ABI Data Collection software v3.0, Sequencing Analysis software 5.2 and SeqScape software v2.5. Sequences are compared to the reference DNA sequence (GenBank Accession: NT_024871.11). Sequencing of a single exon or two exons is available for targeted mutation analysis. Testing includes coverage of the common adult-onset mutation in intron 1 (c.-32-13T>G).

This test was developed and its performance characteristics determined by this laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 ("CLIA") as qualified to perform high complexity clinical testing.

References:

[Duke University Molecular Diagnostics Laboratory](#)
Phone: 919-684-2698 Fax: 919-688-5424