Overview

Useful For
Second-tier testing of newborns with an abnormal primary screening result/decreased acid alpha-glucosidase enzyme for Pompe disease

Follow-up testing for evaluation of an abnormal newborn screening result for Pompe disease

Highlights
This test is used as a second-tier newborn screen for Pompe disease and is based upon a ratio calculated between the creatine (Cre) and creatinine (Crn) ratio and the activity of acid-alpha glucosidase (GAA).

This test can help differentiate true cases of infantile and late onset Pompe disease from false-positive cases (such as carriers and pseudodeficiency of GAA enzyme).

A positive test result supports the utility of follow-up molecular genetic analysis of the GAA gene.

Testing Algorithm
See Newborn Screen Follow-up for Pompe Disease in Special Instructions

For more information, see Newborn Screening Act Sheet Pompe Disease: Decreased Acid Alpha-Glucosidase in Special Instructions.

Special Instructions

- Informed Consent for Genetic Testing
- Biochemical Genetics Patient Information
- Blood Spot Collection Card-Spanish Instructions
- Newborn Screening Act Sheet Pompe Disease: Decreased Acid Alpha-Glucosidase
- Newborn Screen Follow-up for Pompe Disease
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)

Method Name
Flow Injection Analysis-Tandem Mass Spectrometry (FIA-MS/MS)

NY State Available
Yes

Specimen

Specimen Type
Whole blood

Advisory Information
Due to reference range differences, this test is the appropriate test for patients less than or equal to 6 weeks of age. For patients greater than 6 weeks of age, please order PDBS / Pompe Disease, Blood Spot.

Necessary Information
1. Birth weight (grams)
2. Time of birth (24-hour time)
3. Gestational age (weeks)

**Specimen Required**

**Supplies:** Card-Blood Spot Collection (Filter Paper) (T493)

**Container/Tube:**

**Preferred:** Blood Spot Collection Card

**Acceptable:** Ahlstrom 226 filter paper, Munktell filter paper, Whatman Protein Saver 903 paper, or blood collected in tubes containing ACD, EDTA, or heparin and dried on filter paper.

**Specimen Volume:** 3 blood spots

**Collection Instructions:**

1. Let blood dry on the filter paper at ambient temperature in a horizontal position for 3 hours.
2. Do not expose specimen to heat or direct sunlight.
3. Do not stack wet specimens.
4. Keep specimen dry.

**Additional Information:**

1. For collection instructions in Spanish, see [Blood Spot Collection Card-Spanish Instructions](#) (T777) in Special Instructions.
2. For collection instructions in Chinese, see [Blood Spot Collection Card-Chinese Instructions](#) (T800) in Special Instructions.

**Forms**

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available in Special Instructions:
   - [Informed Consent for Genetic Testing](#) (T576)
   - [Informed Consent for Genetic Testing-Spanish](#) (T826)
2. [Biochemical Genetics Patient Information](#) (T602) in Special Instructions
3. If not ordering electronically, complete, print, and send an [Inborn Errors of Metabolism Test Request](#) (T798) with the specimen.

**Specimen Minimum Volume**

1 blood spot

**Reject Due To**
Blood spot | Shows serum rings or has multiple layers

**Specimen Stability Information**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>Refrigerated (preferred)</td>
<td>56 days</td>
<td>FILTER PAPER</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>56 days</td>
<td>FILTER PAPER</td>
</tr>
<tr>
<td></td>
<td>Ambient</td>
<td>7 days</td>
<td>FILTER PAPER</td>
</tr>
</tbody>
</table>

**Clinical and Interpretive**

**Clinical Information**

Pompe disease, also known as glycogen storage disease type II, is an autosomal recessive disorder caused by a deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA; acid maltase) due to mutations in the GAA gene. The estimated incidence is 1 in 40,000 live births. In Pompe disease, glycogen that is taken up by lysosomes during physiologic cell turnover accumulates, causing lysosomal swelling, cell damage and, eventually, organ dysfunction. This leads to progressive muscle weakness, cardiomyopathy, and, eventually, death. Patients with Pompe disease, especially those with infantile, childhood, and juvenile onset, can have elevations of serum enzymes (such as creatine kinase) secondary to cellular dysfunction.

The clinical phenotype of Pompe disease lies on a spectrum, with differing clinical phenotypes dependent on age of onset and residual enzyme activity. Complete loss of enzyme activity causes onset in infancy leading to death, typically within the first year of life when left untreated. Juvenile and adult-onset forms, as the names suggest, are characterized by later onset and longer survival. All disease variants are eventually associated with progressive muscle weakness and respiratory insufficiency. Cardiomyopathy is associated almost exclusively with the infantile form. Treatment with enzyme replacement therapy is available, making early diagnosis of Pompe disease desirable, as early initiation of treatment may improve prognosis. Newborn screening can identify patients with all forms of Pompe disease, even before onset of symptoms. Unaffected patients with GAA pseudodeficiency alleles and carriers may also be identified by newborn screening.

The ratio calculated between the creatine (Cre):creatinine (Crn) ratio as the numerator and the activity of GAA as the denominator can differentiate true cases of infantile and late-onset Pompe disease from false-positive cases (such as carriers and pseudodeficiency of GAA enzyme). When applied to the newborn screening setting, this second-tier testing can provide results in a timely fashion and provide better guidance in the decision to submit samples for further confirmatory testing by molecular genetic analysis (GAAZ / Pompe Disease, Full Gene Analysis).

**Reference Values**

An interpretive report will be provided.

**Interpretation**

An interpretive report will be provided.

The quantitative measurements of informative metabolites and related ratios are evaluated using the Collaborative Laboratory Integrated Reports (CLIR) system. The report is in text form only, indicating if the applicable ratio is normal or abnormal and whether or not the CLIR postanalytical tool is informative for Pompe disease. Abnormal results are not sufficient to conclusively establish a diagnosis of a particular disease. To verify a preliminary diagnosis, independent biochemical (ie, in vitro enzyme assay) or molecular genetic analyses are required, many of
which are offered within Mayo Clinic's Division of Laboratory Genetics and Genomics. Recommendations for additional biochemical testing and confirmatory studies (enzyme assay, molecular analysis) are provided in the interpretative report.

**Cautions**

This test may not detect some late-onset and variant forms of Pompe disease.

Carrier status (heterozygosity) for Pompe disease cannot be reliably detected.

A positive test result is strongly suggestive of a diagnosis but requires follow-up using a stand-alone biochemical or molecular assay, which is best coordinated by local genetics providers.

**Clinical Reference**


**Performance**

**Method Description**

Dried blood spots are processed using 2 analytical protocols with postanalytical integration of all test results.

Protocol 1:

A dried blood spot is extracted by the addition of methanol with known concentrations of isotopically labeled amino acids and acylcarnitines, which are used as internal standards. The extract is derivatized by the addition of 3M HCl in n-butanol. From the residual blood spot a second extraction and derivatization is performed and analyzed concurrently by electrospray tandem mass spectrometry (ESI-MS/MS) for creatine and creatinine. (Turgeon C, Magera M, Allard P, et al: Combined newborns screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clin Chem 2008;54[4]:657-664)

Protocol 2:

Two 3-mm dried blood spots are excised from a single specimen and placed into individual plates. One spot is treated with a solution containing substrate and internal standard for acid sphingomyelinase (ASM), beta-glucocerebrosidase (ABG), alpha-glucosidase (GAA), alpha-galactosidase (GLA), galactocerebrosidase (GALC) and alpha-L-iduronidase (IDUA). The enzyme plate is sealed and incubated overnight. Following the incubation the enzyme plate is purified by liquid-liquid extraction. The second dried blood spot is extracted with methanol containing d4-C26 lysophosphatidylcholines (LPC) on day 2 of the procedure. The LPC extracts and enzyme products are combined and analyzed concurrently ESI-MS/MS. (Tortorelli S, Turgeon C, Gavrilov D, et al: Simultaneous testing for 6 lysosomal storage disorders and X-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. Clin Chem 2016;62[9]:1248-1254)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Saturday; 4 p.m.

Sunday; 1 p.m.
Test Definition: PD2T
Pompe Disease 2ND Tier NBS, BS

Analytic Time
2 days

Maximum Laboratory Time
3 days

Specimen Retention Time
1 year

Performing Laboratory Location
Rochester

Fees and Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
83789

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD2T</td>
<td>Pompe Disease 2ND Tier NBS, BS</td>
<td>63416-2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>48436</td>
<td>Interpretation</td>
<td>63416-2</td>
</tr>
<tr>
<td>48435</td>
<td>Reviewed By</td>
<td>18771-6</td>
</tr>
<tr>
<td>BG700</td>
<td>Birth Weight (grams, XXXX)</td>
<td>8339-4</td>
</tr>
<tr>
<td>BG701</td>
<td>Time of Birth (24hr time, XX:XX)</td>
<td>57715-5</td>
</tr>
<tr>
<td>BG702</td>
<td>Gestational Age (weeks, XX.X)</td>
<td>76516-4</td>
</tr>
</tbody>
</table>