Test Definition: PD2T

Pompe Disease Second-Tier Newborn Screening, Blood Spot

Overview

Useful For

Second-tier testing of newborns with an abnormal primary screening result for Pompe disease (decreased acid alpha-glucosidase enzyme activity)

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

Testing Algorithm

If the patient has abnormal newborn screening result for Pompe disease, immediate action should be taken. Refer to the appropriate ACMG Newborn Screening ACT Sheet.(1)

For more information see Newborn Screen Follow-up for Pompe Disease

Special Instructions

- Informed Consent for Genetic Testing
- Biochemical Genetics Patient Information
- Blood Spot Collection Card-Spanish Instructions
- Newborn Screen Follow-up for Pompe Disease
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

Highlights

This test is used as a second-tier newborn screen for Pompe disease. It is based upon a ratio calculated between the creatine and creatinine 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.

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Ordering Guidance

Due to reference range differences, this is the appropriate test for patients 6 weeks or younger. For patients older than 6 weeks, 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: PerkinElmer 226 filter paper, Munktell filter paper, Whatman Protein Saver 903 paper, local newborn screening card, or blood collected in tubes containing ACD or EDTA and then spotted and dried on filter paper.
Specimen Volume: 3 Blood spots
Collection Instructions:
1. Completely fill at least 3 circles on the filter paper card (approximately 100 microliters blood per circle).
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry.
Additional Information:
1. For collection instructions, see Blood Spot Collection Instructions.
2. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777).
3. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800).

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:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Biochemical Genetics Patient Information (T602)
3. If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request (T798) with the specimen.

Specimen Minimum Volume

1 Blood spot

Reject Due To

Blood spot specimen that	Reject

shows serum rings or has multiple layers	
Insufficient specimen	Reject
Specimens known to have been exposed to elevated temperatures above ambient	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Refrigerated (preferred)	90 days	
	Ambient	28 days	
	Frozen	90 days	

Clinical & 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 variants 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 elevated 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. Newborn screening may also identify unaffected patients with *GAA* pseudodeficiency alleles and carriers.

The ratio calculated using the creatine:creatinine 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 guidance in the decision to submit samples for additional confirmatory testing by molecular genetic analysis (GAAN / Pompe disease, *GAA* Gene Sequencing with Deletion/Duplication, Varies).

Reference Values

An interpretive report will be provided.

Interpretation

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 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 Laboratories. 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 milder or later onset 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

1. ACMG Newborn Screening ACT Sheets Newborn Screening ACT Sheet [Pompe Disease (Glycogen Storage Disease Type II)]. American College of Medical Genetics and Genomics; 2022. Updated July 2022. Accessed October 2, 2025. Available at www.acmg.net/PDFLibrary/Pompe.pdf
2. Pascual JM, Roe CR. Systemic metabolic abnormalities in adult-onset acid maltase deficiency: beyond muscle glycogen accumulation. JAMA Neurol. 2013;70(6):756-763
3. Tortorelli S, Eckerman JS, Orsini JJ, et al. Moonlighting newborn screening markers: The incidental discovery of a second-tier test for Pompe disease. Genet Med. 2018;20(8):840-846. doi:10.1038/gim.2017.190
4. Minter Baerg MM, Stoway SD, Hart J, et al. Precision newborn screening for lysosomal disorders. Genet Med. 2018;20(8):847-854. doi:10.1038/gim.2017.194
5. Ames EG, Fisher R, Kleyn M, Ahmad A. Current practices for U.S. newborn screening of Pompe disease and MPSI. Int J Neonatal Screen. 2020;6(3):72. doi:10.3390/ijns6030072
6. Leslie N, Bailey L. Pompe Disease. In: Adam MP, Feldman J, Mirzaa GM, et al, eds. GeneReviews [Internet]. University of Washington, Seattle; 1993-2025. Updated August 21, 2025. Accessed October 2, 2025. Available at: www.ncbi.nlm.nih.gov/books/NBK1261/

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 containing known concentrations of isotopically labeled amino acids and acylcarnitines, which are used as internal standards. The extract is derivatized by the addition of 3M hydrochloric acid in n-butanol. From the residual blood spot, a second extraction and derivatization are performed and analyzed concurrently by flow injection tandem mass spectrometry 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:
Three 1/8-inch dried blood spots (DBS) are excised from a single specimen. The enzymes are extracted from 2 DBS by incubating the specimens with a mix of substrate and internal standard for acid sphingomyelinase, beta-glucocerebrosidase, alpha-glucosidase, alpha-galactosidase, galactocerebrosidase, alpha-L-iduronidase, and iduronate 2-sulfatase. The sample is then purified by liquid-liquid extraction. The third DBS is extracted with methanol containing d4-C26 lysophosphatidylcholines. The resulting extracts are then combined, evaporated, and reconstituted before analysis by tandem mass spectrometry. (Tortorelli S, Turgeon CT, Gavrilov DK, 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) Performed
Monday through Saturday

Report Available
2 to 3 days

Specimen Retention Time
6 months

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

83789

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PD2T	Pompe Disease 2ND Tier NBS, BS	63416-2

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