MAYO CLINIC LABORATORIES

Test Definition: KD2T

Krabbe Disease Second-Tier Newborn Screen, Blood Spot

Overview

Useful For

Second-tier testing of newborns with an abnormal screening result for Krabbe disease

Follow-up testing after an abnormal newborn screening result for Krabbe disease

Genetics Test Information

This test is used as a second-tier newborn screen for Krabbe disease (galactocerebrosidase deficiency) and includes both psychosine measurement and DNA analysis for the 30-kb deletion.

Testing Algorithm

For more information, see Newborn Screening Act Sheet Krabbe Disease: Decreased Galactocerebrosidase in Special Instructions.

Special Instructions

- Informed Consent for Genetic Testing
- Biochemical Genetics Patient Information
- Blood Spot Collection Card-Spanish Instructions
- Newborn Screening Act Sheet Krabbe Disease: Decreased Galactocerebrosidase
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

Highlights

Krabbe disease (globoid cell leukodystrophy) is an autosomal recessive lysosomal storage disorder caused by an enzyme deficiency of galactocerebrosidase (GALC).

Although Krabbe disease is clinically variable, the most common and severe form of the disorder is early infantile onset that presents with rapid neurological regression and results in early death.

Second-tier testing reduces the number of false-positive results reported out.

Elevations in psychosine or the presence of a homozygous 30 kilobase deletion of the *GALC* gene support a diagnosis of Krabbe disease.

Method Name

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)/Polymerase Chain Reaction with Gel Electrophoresis

NY State Available

Yes

Specimen

Specimen Type
Whole blood

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)
Collection Container/Tube:
Preferred: Blood Spot Collection Card
Acceptable: PerkinElmer 226 (formerly Ahlstrom 226) filter paper, Munktell filter paper, Whatman Protein Saver 903 paper, or blood collected in tubes containing heparin or EDTA and dried on filter paper.
Specimen Volume: 3 Blood spots
Collection Instructions:
1. An alternative blood collection option for a patient older than 1 year of age is fingerstick. See Dried Blood Spot Collection Tutorial for how to collect blood spots via fingerstick: https://vimeo.com/508490782 .
2. Completely fill at least 3 circles on the filter paper card (approximated 100-microliters blood per circle).
3. Let blood dry on filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
4. Do not expose specimen to heat or direct sunlight.
5. Do not stack wet specimens.
6. Keep specimen dry.
Additional Information:
1. For collection instructions, see Blood Spot Collection Instructions in Special Instructions.
2. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777) in Special Instructions.
3. 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 a Biochemical Genetics Test Request (T798) with the specimen.

Specimen Minimum Volume
Blood spot: 2

Reject Due To

Shows serum rings Insufficient specimen Layering Multiple applications	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	96 days	FILTER PAPER
	Frozen	96 days	FILTER PAPER
	Refrigerated	96 days	FILTER PAPER

Clinical & Interpretive

Clinical Information

Krabbe disease (globoid cell leukodystrophy) is an autosomal recessive disorder caused by a deficiency of galactocerebrosidase leading to an accumulation of galactosylceramide and severe demyelination throughout the brain. Krabbe disease is primarily caused by variants in the *GALC* gene, and it has an estimated frequency of 1 in 100,000 births.

The clinical course of Krabbe disease can be variable, even within the same family. Eighty-five percent to 90% of patients present before the first year of life with central nervous system impairment including increasing irritability, developmental delay, and sensitivity to stimuli. Rapid neurodegeneration including white matter disease is followed by death usually by age 2. Late onset forms of the disease affect 10% to15% of individuals and are characterized by ataxia, vision loss, weakness, and psychomotor regression typically presenting from age 6 months to the seventh decade of life.

Newborn screening for Krabbe disease recently has been implemented in some states. The early (presymptomatic) identification and subsequent testing of infants at risk for Krabbe disease may be helpful in reducing the morbidity and mortality associated with this disease. While treatment is mostly supportive, hematopoietic stem cell transplantation has shown some success if performed prior to onset of neurologic damage.

Newborn screening can typically identify patients with Krabbe disease, even before onset of symptoms, as well as unaffected patients with *GALC* pseudodeficiency alleles. For these reasons, second-tier testing that includes both psychosine and 30-kilobase (kb) deletion analyses has been developed. Second-tier testing reduces the number of false-positive results and limits the identification of affected individuals to patients needing immediate follow-up.

Psychosine (PSY), a neurotoxin at elevated concentrations, is 1 of 4 substrates degraded by galactocerebrosidase. It has

been shown to be elevated in patients with active disease and, therefore, may be a useful biomarker for the presence of disease or disease progression.

The common 30-kb deletion spanning intron 10 through the end of the gene accounts for a significant proportion of disease alleles that contribute to infantile Krabbe disease. While enzyme activity alone is not predictive of age of onset, there are known genotype-phenotype correlations. Individuals who are homozygous for the deletion or compound heterozygous for the deletion and a second *GALC* genetic variant (with the exception of late-onset genetic variants) are predicted to have infantile Krabbe disease.

Although rare, a few infants with an early onset Krabbe disease phenotype due to deficiency of saposin A (SAP-A) have been identified. SAP-A is a sphingolipid activator protein that assists galactocerebrosidase in its action on galactosylceramide.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided.

An elevation of psychosine is indicative of symptomatic Krabbe disease.

The presence of a homozygous 30-kilobase deletion is indicative of early onset Krabbe disease.

Cautions

The absence of the 30-kilobase deletion in the *GALC* gene does not eliminate the possibility of positive-carrier status or the diagnosis of Krabbe disease. This assay does not include DNA sequencing of the *GALC* gene.

A Krabbe disease phenotype can also be caused by the absence of a physiologically active sphingolipid activator protein, saposin A.

Psychosine levels may be normal in patients who are not yet symptomatic or have late onset Krabbe disease.

Clinical Reference

1. Turgeon CT, Orsini JJ, Sanders KA, et al: Measurement of psychosine in dried blood spots-a possible improvement to newborn screening programs for Krabbe disease. J Inherit Metab Dis. 2015 Sep;38(5):923-929
2. Orsini J, Morrissey M, Slavin L, et al: Implementation of newborn screening for Krabbe disease: Population study and cutoff determination. Clin Biochem. 2009;42:877-884
3. Orsini JJ, Escolar ML, Wasserstein MP, et al: Krabbe disease. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. GeneReviews [Internet]. University of Washington, Seattle; 2000. Updated October 11, 2018. Accessed June 3, 2021. Available at www.ncbi.nlm.nih.gov/books/NBK1238/
4. Wenger DA, Escolar ML, Luzi P, Rafi MA: Krabbe disease (globoid cell leukodystrophy). In: Valle DL, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA. eds. The Online Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill; 2019. Accessed June 03, 2021. Available at https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225546481

Performance

Method Description

Protocol 1:
Internal standard is added to a dried blood spot. The extract is evaporated and reconstituted prior to injection onto a liquid chromatography-tandem mass spectrometry (LC-MS/MS). Following separation of the structural isomers glucopsychosine (GPSY) and psychosine by liquid chromatography, their concentrations are measured by MS/MS analysis in the multiple reaction monitoring positive mode to follow the precursor to product species transitions for psychosine (PSY). The ratio of the extracted peak area of PSY to internal standard as determined by LC-MS/MS is used to calculate the concentration of PSY in the sample.(Unpublished Mayo method)

Protocol 2:
A polymerase chain reaction-based assay is used to examine DNA for the presence of a 30-kilobase deletion encompassing exon 11 through the end of the *GALC* gene.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

1 to 2 days

Specimen Retention Time

6 months

Performing Laboratory Location

Rochester

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. This test has not been cleared or approved by the US Food and Drug Administration.

Test Definition: KD2T

Krabbe Disease Second-Tier Newborn Screen, Blood Spot

CPT Code Information

82542-Psychosine
81401-30-kb deletion

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
KD2T	Krabbe Disease 2ND Tier NBS, BS	62309-0

Result ID	Test Result Name	Result LOINC® Value
48536	Interpretation	62309-0
48535	Reviewed By	18771-6
BG704	Birth Weight (grams, XXXX)	8339-4
BG705	Time of Birth (24hr Time, XX:XX)	57715-5
BG706	Gestational Age (weeks, XX.X)	76516-4