

Fabry Disease, GLA Gene Sequencing with Deletion/Duplication, Varies

Overview

Useful For

Confirmation of a diagnosis of classic or variant Fabry disease in affected male patients with reduced alpha-galactosidase A enzyme activity

Carrier or diagnostic testing for asymptomatic or symptomatic female patients, respectively

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
_STR1	Comp Analysis using STR	No, (Bill only)	No
	(Bill only)		
_STR2	Add'l comp analysis w/STR	No, (Bill only)	No
	(Bill Only)		
CULFB	Fibroblast Culture for	Yes	No
	Genetic Test		
CULAF	Amniotic Fluid	Yes	No
	Culture/Genetic Test		
MATCC	Maternal Cell	Yes	No
	Contamination, B		

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *GLA* gene associated with Fabry disease. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for Fabry disease.

Testing Algorithm

Prenatal specimens only:

If an amniotic fluid specimen or cultured amniocytes are received, an amniotic fluid culture will be performed at an additional charge.

If a chorionic villi specimen or cultured chorionic villi are received, a fibroblast culture will be performed at an additional charge.

For any prenatal specimen that is received, maternal cell contamination testing will be performed at an additional charge.

Skin biopsy or cultured fibroblast specimens:

For skin biopsy or cultured fibroblast specimens, a fibroblast culture will be performed at an additional charge.



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For information see <u>Hereditary Peripheral Neuropathy Diagnostic Algorithm</u>

Special Instructions

- Informed Consent for Genetic Testing
- Hereditary Peripheral Neuropathy Diagnostic Algorithm
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing.

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Biochemical testing for the diagnosis of Fabry disease should be considered prior to molecular confirmation with this test. For the recommended first-tier test for male patients with suspected Fabry disease, see either AGAW / Alpha-Galactosidase, Leukocytes; or AGAS / Alpha-Galactosidase, Serum.

Testing for the *GLA* gene as part of a customized panel is available. See CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies. To modify this panel via CGPH, please use the Inborn Errors of Metabolism disease state for step 1 on the <u>Custom Gene Ordering Tool</u>.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for variants identified in the *GLA* gene. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Additional Testing Requirements

All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen as this must be a different order number than the prenatal specimen.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing.



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For information about for testing patients who have received a hematopoietic stem cell transplant, call 800-533-1710

Submit only 1 of the following specimens:

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA) or yellow top (ACD)

Specimen Volume: 3 mL **Collection Instructions**:

1. Invert several times to mix blood.

2. Send whole blood specimen in original tube. **Do not aliquot**.

3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information

Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days

Additional Information:

- 1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
- 2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
- 3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Saliva

Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.

Supplies:

DNA Saliva Kit High Yield (T1007) Saliva Swab Collection Kit (T786)

Container/Tube:

Preferred: High-yield DNA saliva kit

Acceptable: Saliva swab

Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786 **Collection Instructions:** Collect and send specimen per kit instructions.

Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days

Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Specimen Type: Skin biopsy

Supplies: Fibroblast Biopsy Transport Media (T115)

Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The

solution should be supplemented with 1% penicillin and streptomycin.

Specimen Volume: 4-mm Punch

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours



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Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts

Source: Skin

Container/Tube: T-25 flask Specimen Volume: 2 Flasks

Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy. **Specimen Stability Information**: Ambient (preferred) < 24 hours/Refrigerated < 24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Tissue biopsy Supplies: Hank's Solution (T132)

Container/Tube: Sterile container with sterile Hank's balanced salt solution, Ringer's solution, or normal saline

Specimen Volume: 0.5 to 3 cm(3) or larger

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Blood spot

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

Container/Tube:

Preferred: Collection card (Whatman Protein Saver 903 Paper)

Acceptable: PerkinElmer 226 filter paper or blood spot collection card

Specimen Volume: 2 to 5 Blood spots

Collection Instructions:

- 1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect a Dried Blood Spot Sample.
- 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.

Specimen Stability Information: Ambient (preferred)/Refrigerated

Additional Information:



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- 1. Blood spot specimens are acceptable but not recommended. Multiple extractions will be required to obtain sufficient yield for supplemental analysis, and there is significant risk for test failure due to insufficient DNA.
- 2. Due to lower concentration of DNA yielded from blood spot, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.
- 3. For collection instructions, see <u>Blood Spot Collection Instructions</u>
- 4. For collection instructions in Spanish, see <u>Blood Spot Collection Card-Spanish Instructions</u> (T777)
- 5. For collection instructions in Chinese, see <u>Blood Spot Collection Card-Chinese Instructions</u> (T800)

Specimen Type: Extracted DNA

Container/Tube:

Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base

Acceptable: Matrix tube, 1 mL

Collection Instructions:

- 1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
- 2. Include concentration and volume on tube.

Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated

Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

PRENATAL SPECIMENS

Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.

Specimen Type: Amniotic fluid

Container/Tube: Amniotic fluid container

Specimen Volume: 20 mL

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information: Specimen will only be tested after culture.

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks are required to culture amniotic fluid before genetic testing can occur.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Prenatal cultured fibroblasts (eg, products of conception), amniocytes, or other confluent cultured cells. This does not include cultured chorionic villi.

Container/Tube: T-25 flask Specimen Volume: 2 Flasks



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Collection Instructions: Submit confluent cultured cells from another laboratory. **Specimen Stability Information**: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Chorionic villi

Container/Tube: 15-mL tube containing 15 mL of transport media

Specimen Volume: 20 mg

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information: Specimen will only be tested after culture.

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Cultured chorionic villi

Container/Tube: T-25 flasks Specimen Volume: 2 full flasks

Collection Instructions: Submit confluent cultured cells from another laboratory. **Specimen Stability Information**: Ambient (preferred) < 24 hours/Refrigerated < 24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

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. Molecular Genetics: Biochemical Disorders Patient Information (T527)
- 3. If not ordering electronically, complete, print, and send a <u>Biochemical Genetics Test Request</u> (T798) with the specimen.



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Specimen Minimum Volume

See Specimen Required

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Note: Where applicable, verbiage refers to sex assigned at birth.

Fabry disease is an X-linked recessive disorder with an incidence of approximately 1 in 50,000 male individuals. Symptoms result from a deficiency of the enzyme alpha-galactosidase A (alpha-Gal A), encoded on the *GLA* gene. Reduced alpha-Gal A activity results in accumulation of glycosphingolipids in the lysosomes of both peripheral and visceral tissues.

Severity and onset of symptoms are dependent on the residual alpha-Gal A activity. Male patients with less than 1% alpha-Gal A activity have the classic form of Fabry disease. Symptoms can appear in childhood or adolescence and usually include acroparesthesias (pain crises), multiple angiokeratomas, reduced or absent sweating, and corneal opacity. By middle age, most patients develop kidney insufficiency leading to kidney failure, as well as cardiac and cerebrovascular disease. Male patients with greater than 1% alpha-Gal A activity may present with a variant form of Fabry disease. The renal variant generally has onset of symptoms in the third decade. The most prominent feature in this form is kidney insufficiency and, ultimately, kidney failure. Individuals with the renal variant may or may not have other symptoms of classic Fabry disease. Individuals with the cardiac variant are often asymptomatic until they present with cardiac findings, such as cardiomyopathy or mitral insufficiency, later in life. The cardiac variant is not associated with kidney failure.

Female heterozygotes of Fabry disease can have a broad spectrum of clinical presentations ranging from asymptomatic to severe. Measurement of alpha-Gal A activity is not recommended to detect Fabry disease heterozygotes, as many of these individuals have normal levels of alpha-Gal A.

Most of the *GLA* variants identified to date are family specific. Full sequencing of the *GLA* gene identifies over 98% of the sequence variants in the coding region and splice junctions. In addition, this assay detects the intron 4 variant common in the Taiwanese population.(1)

The recommended first-tier test for male patients with suspected Fabry disease is biochemical testing that measures alpha-galactosidase enzyme activity in blood or serum: AGAW / Alpha-galactosidase, Leukocytes or AGAS / Alpha-galactosidase, Serum. Additionally, testing for the glycosphingolipid, globotriaosylsphingosine (LGb3) may aid in



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further clarifying disease status in both male and female patients with suspected Fabry disease (LGB3 / Globotriaosylsphingosine, Serum). Individuals with decreased or absent enzyme activity and elevated LGb3 are more likely to have identifiable variants in the *GLA* gene by molecular genetic testing. However, enzymatic testing alone is not reliable to detect heterozygotes.

The following algorithms are available:

- -Fabry Disease: Newborn Screen-Positive Follow-up algorithm
- -Fabry Disease Diagnostic Testing Algorithm

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(1) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:

Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of the gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution



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cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic mutations and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent non-leukoreduced blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:

Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare professionals to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:

Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline. (2) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. Incidental findings may include, but are not limited to, results related to the sex chromosomes. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

- 1. Hwu WL, Chien YH, Lee NC, et al. Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset GLA mutation c.936+919G>A). Hum Mutat. 2009:30(10):1397-1405
- 2. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424
- 3. Germain DP. Fabry disease. Orphanet J Rare Dis. 2010;22;5:30



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4 Wang RY, Lelis A, Mirocha J, Wilcox WR. Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life. Genet Med. 2007;9(1):34-35

5. Henderson N, Berry L, Laney DA. Fabry disease practice resource: Focused revision. J Genet Couns. 2020;29(5):715-717

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of variants in coding regions and intron/exon boundaries of the *GLA* gene, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 20X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction based quantitative method is performed to test for the presence of deletions and duplications in the gene analyzed.

There may be regions of the gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. (Unpublished Mayo method)

The reference transcript for the *GLA* gene is NM_000169.2. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

The following additional noncoding variants are being analyzed by this test: c.640-801G>A, c.640-830T>C and c.640-859C>T.

PDF Report

Supplemental

Day(s) Performed

Varies

Report Available

10 to 14 days

Specimen Retention Time

Whole blood/Saliva: 28 days (if available); Extracted DNA: 3 months; Blood spots: 1 year (if available)

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus



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Fees & Codes

Fees

- Authorized users can sign in to <u>Test Prices</u> for detailed fee information.
- Clients without access to Test Prices can contact <u>Customer Service</u> 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact <u>Customer Service</u>.

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

81405-GLA (galactosidase, alpha) (eg, Fabry disease), full gene sequence 88233-Tissue culture, skin, or solid tissue biopsy (if appropriate) 88240-Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
GLA	GLA Gene Analysis	76036-3

Test Result Name	Result LOINC® Value
Test Description	62364-5
Specimen	31208-2
Source	31208-2
Result Summary	50397-9
Result	82939-0
Interpretation	69047-9
Additional Results	82939-0
Resources	99622-3
Additional Information	48767-8
Method	85069-3
Genes Analyzed	48018-6
Disclaimer	62364-5
Released By	18771-6
	Test Description Specimen Source Result Summary Result Interpretation Additional Results Resources Additional Information Method Genes Analyzed Disclaimer