

Mitochondrial Full Genome Analysis, Next-Generation Sequencing (NGS), Varies

#### Overview

#### **Useful For**

Diagnosis of the subset of mitochondrial diseases that result from variants in the mitochondrial genome

A second-tier test for patients in whom previous targeted gene variant analyses for specific mitochondrial disease-related genes were negative

Identifying variants within genes of the mitochondrial genome that are known to be associated with mitochondrial disease, allowing for predictive testing of at-risk family members

#### **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 uses next-generation sequencing (NGS) to test for the presence of variants, including 13 protein coding genes, 22 transfer RNA genes, and 2 ribosomal RNA genes, within the mitochondrial genome.

Large deletions within the mitochondrial genome and their locations are determined from the NGS data and confirmed by droplet digital polymerase chain reaction (ddPCR). Large deletion heteroplasmy level is also determined by ddPCR.

This test is only useful for detecting mitochondrial genomic variants. Depletion of mitochondrial DNA levels or variants in mitochondrial genes encoded by the nuclear genome is not within the scope of this test.

#### **Testing Algorithm**

#### Skin biopsy:

If skin biopsy is received, fibroblast culture will be added at an additional charge. If viable cells are not obtained, the client will be notified.

### **Prenatal specimens:**

If an amniotic fluid specimen is received, an amniotic fluid culture will be performed at an additional charge.



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If chorionic villi, cultured chorionic villi, or cultured amniocyte specimen is 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.

#### Cord blood:

For cord blood specimens that have an accompanying maternal blood specimen, maternal cell contamination studies will be performed at an additional charge.

The following algorithms are available:

- -Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm
- -Neuromuscular Myopathy Testing Algorithm

### **Special Instructions**

- Muscle Biopsy Specimen Preparation Instructions
- Molecular Genetics: Biochemical Disorders Patient Information
- Informed Consent for Genetic Testing
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm
- Neuromuscular Myopathy Testing Algorithm
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

#### **Method Name**

Long-Range Polymerase Chain Reaction (LR-PCR) followed by Next-Generation Sequencing (NGS) and Droplet Digital Polymerase Chain Reaction (ddPCR) as needed

## NY State Available

Yes

## **Specimen**

## Specimen Type

Varies

### **Ordering Guidance**

If testing for variants in the mitochondrial genes encoded by the nuclear genome is requested, order NMITO / Nuclear Mitochondrial Gene Panel, Next-Generation Sequencing, Varies. Alternatively, order CMITO / Combined Mitochondrial Full Genome and Nuclear Gene Panel, Varies for both the mitochondrial genome and mitochondrial genes encoded by the nuclear genome.



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### **Additional Testing Requirements**

**For cord blood specimens**: Maternal cell contamination (MCC) studies are available. **Order MATCC /** Maternal Cell Contamination, Molecular Analysis, Varies **on both the cord blood and maternal specimens under separate order numbers**. Cord blood testing will proceed without MCC studies, but results may be compromised if MCC is present.

### **Necessary Information**

Molecular Genetics: Biochemical Disorders Patient Information (T527) is available to provide information useful for accurate test interpretation. At minimum, provide a reason for testing with each specimen. Although testing may proceed without this information, ordering healthcare professionals are strongly encouraged to complete the form and send it with the specimen.

#### Specimen Required

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

Preferred: Lavender top (EDTA) or yellow top (ACD)

Acceptable: Green top (Sodium heparin)

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 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.



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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

#### **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 tissue 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**: Snap frozen nerve tissue biopsy

Collection Instructions: Prepare snap frozen tissue biopsy per surgical procedure

**Specimen Volume**: 0.25 to 0.5 cm **Specimen Stability Information**: Frozen



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**Specimen Type**: Muscle tissue biopsy **Supplies**: Muscle Biopsy Kit (T541) **Specimen Volume**: 20 to 80 mg

**Collection Instructions**: Prepare and transport specimen per instructions in <u>Muscle Biopsy Specimen Preparation</u>. **Specimen Stability Information**: Frozen (preferred) <24 hours/Ambient <24 hours/Refrigerated <24 hours **Additional Information**: Specimens are preferred to be received within 24 hours of collection. Extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.

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:**

- 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.
- 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



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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: Confluent cultured amniocytes

Container/Tube: T-25 flask Specimen Volume: 2 Full flasks

**Collection Instructions**: Submit confluent cultured amniocytes 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.
- 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 is 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.



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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.
- 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 1 of the following forms with the specimen:
- -Neurology Specialty Testing Client Test Request (T732)
- -Biochemical Genetics Test Request (T798)

### **Specimen Minimum Volume**

See Specimen Required

### Reject Due To

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

## **Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

## Clinical & Interpretive

#### **Clinical Information**

The mitochondrion occupies a unique position in eukaryotic biology. It is the site of energy metabolism, and it is the sole subcellular organelle composed of proteins derived from 2 genomes, mitochondrial and nuclear. A group of hereditary disorders due to variants in either the mitochondrial genome or nuclear mitochondrial genes have been well characterized.

The diagnosis of mitochondrial disease can be particularly challenging as the presentation can occur at any age, involve virtually any organ system, and be associated with widely varying severities. This test utilizes massively parallel sequencing, also termed next-generation sequencing (NGS), to determine the exact sequence of the entire 16,569



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base-pair mitochondrial genome.

The utility of this test is to assist in the diagnosis of the subset of mitochondrial diseases that result from variants in the mitochondrial genome. This includes certain types of myopathies and neuro-ophthalmologic diseases, such as MELAS (mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibers), mitochondrial myopathy, neurogenic muscle weakness, ataxia, retinitis pigmentosa, Leigh syndrome, Leber hereditary optic neuropathy, and chronic progressive external ophthalmoplegia. In addition to the detection of single base changes with these disorders, large deletions, such as those associated with Kearns-Sayre or Pearson syndromes, are also detected. Variants in mitochondrial proteins encoded by genes in the nucleus, such as the enzymes of fatty acid oxidation, are not detected using this test.

In contrast to variants in nuclear genes, which are present in either 0, 1, or 2 copies, mitochondrial variants can be present in any fraction of the total organelles, a phenomenon known as heteroplasmy. Typically, the severity of disease presentation is a function of the degree of heteroplasmy. Individuals with a higher fraction of altered mitochondria present with more severe disease than those with lower percentages of altered alleles. The sensitivity for the detection of altered alleles in a background of wild-type (or normal) mitochondrial sequences by NGS is approximately 10%.

#### **Reference Values**

An interpretive report will be provided.

#### Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics guidelines for mitochondrial DNA variant interpretation.(1) 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. The degree of heteroplasmy of each single nucleotide or delin (deletion-insertion) variant, defined as the ratio (percentage) of variant sequence reads to the total number of reads, will also be reported. Variants detected at or above 95% will be reported as homoplasmic. Heteroplasmy for large deletions will be reported and is determined by droplet digital polymerase chain reaction. Variants classified as benign or likely benign are not reported.

#### Cautions

#### **Clinical Correlations:**

A small percentage of individuals who have mitochondrial genome involvement may have a variant that is not identified by the methods performed. The absence of a variant, therefore, does not eliminate the possibility of a mitochondrial disease due to variant in the mitochondrial genome. Variants in mitochondrial genes encoded by the nuclear genome will not be detected with this assay. For predictive testing of asymptomatic individuals, it is important to first document the presence of a gene variant in an affected family member.

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.

#### **Technical Limitations:**

In some cases, DNA variants of undetermined significance may be identified.

Rare variants (ie, polymorphisms) exist that could lead to false-negative or false-positive results. If results obtained do



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not match the clinical findings, additional testing should be considered.

#### **Evaluation Tools:**

Multiple in-silico evaluation tools were used to assist in the interpretation of these results. These tools are updated regularly; therefore, changes to these algorithms may result in different predictions for a given variant. Additionally, the predictability of these tools for the determination of pathogenicity is currently unvalidated.

Unless reported or predicted to cause disease, variants in protein coding genes that do not result in an amino acid substitution are not reported. These and common variants identified for this patient are available upon request.

#### Reclassification of Variants-Policy:

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.

#### **Clinical Reference**

- 1. McCormick EM, Lott MT, Dulik MC, et al. Specifications of the ACMG/AMP standards and guidelines for mitochondrial DNA variant interpretation. Hum Mutat. 2020;41(12):2028-2057
- 2. Munnich A, Rotig A, Cormier-Daire V, Rustin P. Clinical presentation of respiratory chain deficiency. In: Valle D, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA, eds. The Online Metabolic and Molecular Basis of Inherited Disease. McGraw-Hill; 2019. Accessed June 16, 2025. Available at

https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225086827

3. Wallace DC, Lott MT, Brown MD, Kerstann K. Mitochondria and neuro-ophthalmologic diseases. In: Valle D, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA, eds. The Online Metabolic and Molecular Basis of Inherited Disease. McGraw-Hill; 2019. Accessed June 16, 2025. Available at

https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225088522

4. Wong LJ. Molecular genetics of mitochondrial disorders. Dev Disabil Res Rev. 2010;16(2):154-162. doi:10.1002/ddrr.104

#### **Performance**

#### **Method Description**

Next-generation sequencing (NGS) is used to test for the presence of variants within the mitochondrial genome (includes 13 protein coding genes, 22 transfer RNA genes, and 2 ribosomal RNA genes) and to determine the mitochondrial haplogroup of the patient. Large deletions within the mitochondrial genome are first detected by gel electrophoresis (as size-shifted polymerase chain reaction bands), and the locations of the deletions in the mitochondrial DNA are then determined from the NGS data. Droplet digital polymerase chain reaction (ddPCR) is utilized to confirm the presence of large deletions and determine heteroplasmy level.(Unpublished Mayo method)

The haplogroup is computed using the software package HaploGrep and PhyloTree. (Weissensteiner H, Pacher D,



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Kloss-Brandstatter A, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. Nucleic Acids Res. 2016;44[W1]:W58-W63. doi:10.1093/nar/gkw233; van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009;30[2]:E386-E394. doi:10.1002/humu.20921. Available at www.phylotree.org)

#### PDF Report

No

### Day(s) Performed

**Varies** 

#### Report Available

28 to 42 days

#### **Specimen Retention Time**

Whole blood: 28 days (if available); Saliva: 30 days (if available); Extracted DNA: 3 months; Blood spots: 1 year (if available)

### **Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Main Campus

### **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 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**

81460-Whole Mitochondrial Genome 81465-Whole Mitochondrial Genome Large Deletion Analysis 88233-Tissue culture, skin, solid tissue biopsy (if appropriate) 88240-Cryopreservation (if appropriate)

#### **LOINC®** Information

Test ID	Test Order Name	Order LOINC® Value
MITOP	Mitochondrial Full Genome Analysis	101152-7

Result ID	Test Result Name	Result LOINC® Value
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55281	Result Summary	50397-9
55282	Result	82939-0
55283	Interpretation	69047-9
55284	Additional Information	48767-8
55285	Specimen	31208-2
55286	Source	31208-2
55287	Released By	18771-6