

## Overview

### Useful For

Serving as a first-tier test to identify a molecular diagnosis in patients with suspected genetic disorders, which can allow for:

- Better understanding of the natural history/prognosis
- Targeted management (anticipatory guidance, management changes, specific therapies)
- Predictive testing of at-risk family members
- Testing and exclusion of disease in siblings or other relatives
- Recurrence risk assessment

Serving as a second-tier test for patients in whom previous genetic testing was negative

Providing a potentially cost-effective alternative to establishing a molecular diagnosis compared to performing multiple independent molecular assays(1)

### Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No
G227	Number of Comparators for WGSDX	No, (Bill Only)	No
MATCC	Maternal Cell Contamination, B	Yes	No
CULFB	Fibroblast Culture for Genetic Test	Yes	No

### Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide variants, small insertions and deletions, copy number variants, mitochondrial genome variants, and select repeat expansion variants throughout the genome. 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.

It is highly recommended that specimens are submitted from the patient (proband), the patient's biological mother, and

the patient's biological father (trio analysis). However, testing for the patient only (singleton), the patient and one first-degree relative (duo), or the patient and two first-degree relatives (nontraditional trios) will also be accepted if the patient's biological mother and biological father are not available for testing. Testing typically includes up to two family member comparators. Contact the laboratory for approval to send the patient and three first-degree relatives (quad).

## Testing Algorithm

If a cord blood specimen is received, maternal cell contamination testing will be added and performed at an additional charge.

## Skin biopsy or cultured fibroblast specimens:

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

## Special Instructions

- [Muscle Biopsy Specimen Preparation Instructions](#)
- [Hereditary Peripheral Neuropathy Diagnostic Algorithm](#)
- [Whole Genome Sequencing: Ordering Checklist](#)
- [Whole Exome and Genome Sequencing Information and Test Ordering Guide](#)

## Highlights

Additional information is available; see [Whole Exome and Genome Sequencing Information and Test Ordering Guide](#).

## Method Name

Next-Generation Sequencing (NGS) followed by Sanger Sequencing, Quantitative Polymerase Chain Reaction (qPCR), or other methods, as needed.

## NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Ordering Guidance

The American College of Medical Genetics and Genomics recommends that whole genome sequencing be considered as a first-tier or second-tier test for patients with one or more congenital anomalies, or developmental delay or intellectual disability with age of onset prior to 18 years.(1)

If a specific diagnosis is suspected, single gene or panel testing may be a more appropriate first-tier testing option.

**This test is for affected patients (probands) only.** For family member specimens being sent as comparators, order

CMPRG / Family Member Comparator Specimen for Genome Sequencing, Varies. If this test is ordered on a family member comparator, this test will be canceled and CMPRG performed as the appropriate test.

This test is **not appropriate** for identification of somatic variants in solid tumors, other malignancies, or other abnormal tissues. Multiple oncology (cancer) gene panels are available. For more information see [Hematology, Oncology, and Hereditary Test Selection Guide](#). If testing for other malignancies is needed, contact the laboratory for test selection guidance.

This testing does not provide genotyping of patients for pharmacogenomic purposes. For an assessment of genes with strong drug-gene associations, order PGXQP / Focused Pharmacogenomics Panel, Varies.

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

**Prenatal specimens (amniocentesis or chorionic villi) are not currently accepted for this test.**

### Additional Testing Requirements

To order whole genome sequencing for the patient and the family member comparator specimens, see the following steps:

1. Order this test (WGSDX) on the patient (proband).
2. Order CMPRG / Family Member Comparator Specimen for Genome Sequencing, Varies on all family members' specimens being submitted as comparators.
  - a. When available, the patient's biological mother and biological father are the preferred family member comparators.
  - b. If one or both of the patient's biological parents are not available for testing, specimens from other first-degree relatives (siblings or children) can be used as comparators. Testing typically includes up to two family member comparators. Contact the laboratory at 800-533-1710 for approval to send specimens from other relatives or to send the patient and three first-degree relatives (quad).
  - c. The cost of analysis for family member comparator specimens is applied to the patient's (proband's) test. Family members will not be charged separately.
3. Collect patient (proband) and family member specimens. Label specimens with full name and birthdate. **Do not** label family members' specimens with the proband's name.
4. For each family, complete the following portions of the [Whole Genome Sequencing: Ordering Checklist](#). **A separate form is not needed for each family member.**
  - a. **Patient Information is required for all clients.**
  - b. Informed Consent is **required for New York State clients.**
  - c. **If the patient wishes to opt-out of receiving secondary findings or change the DNA storage selection**, select the appropriate boxes in the Informed Consent section.
5. Attach clinic notes from specialists relevant to patient's clinical features, if available.
6. Attach pedigree, if available.
7. Send paperwork to the laboratory along with the specimens. If not sent with the specimens, fax a copy of the paperwork to 507-284-1759, Attn: WGS Genetic Counselors.

For more information see [Whole Exome and Genome Sequencing Information and Test Ordering Guide](#).

## Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

## Necessary Information

[Whole Genome Sequencing: Ordering Checklist](#) is required for all patients, and Informed Consent is required for New York clients. Fill out one form for the family and send with the specimens.

## Specimen Required

**Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710.

**Submit only 1 of the following specimens for each family member.**

**Specimen Type:** Whole blood

**Container/Tube:**

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

**Acceptable:** Any anticoagulant

**Specimen Volume:** 3 mL

**Collection Instructions:**

1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**

**Specimen Stability Information:** Ambient (preferred)/Refrigerated

**Additional Information:**

1. If a cord blood specimen is received, MATCC / Maternal Cell Contamination, Molecular Analysis, Varies will be performed at an additional charge; maternal blood sample is required.
2. To ensure minimum volume and concentration of DNA is met, the preferred volume of blood must be submitted. Testing may be canceled if DNA requirements are inadequate.

**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:** Refrigerated (preferred)/Ambient

**Additional Information:** 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.

**Specimen Type:** Cultured fibroblast

**Container/Tube:** T-25 flask

**Specimen Volume:** 2 Flasks

**Collection Instructions:** Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.

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

**Additional Information:** 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.

**Specimen Type:** Saliva

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

**Supplies:** Saliva Swab Collection Kit (T786)

**Specimen Volume:** 1 Swab

**Collection Instructions:** Collect and send specimen per kit instructions.

**Specimen Stability Information:** Ambient 30 days

**Additional Information:** 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:** Muscle tissue biopsy

**Supplies:** Muscle Biopsy Kit (T541)

**Collection Instructions:** Prepare and transport specimen per instructions in [Muscle Biopsy Specimen Preparation Instructions](#).

**Specimen Volume:** 10 to 80 mg

**Specimen Stability Information:** Frozen (preferred)/Ambient/Refrigerated

## Forms

- [Whole Genome Sequencing: Ordering Checklist](#) is required
- New York Clients-Informed consent is required and is included in the above form.** Document on the request form or electronic order that a copy is on file.
- 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

Whole blood: 1 mL; Other specimen types: 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	Ambient (preferred)		
	Frozen		
	Refrigerated		

## Clinical & Interpretive

## Clinical Information

This test uses next-generation sequencing technology to assess the genome of patients with suspected underlying genetic disorders. Indications for whole genome sequencing include, but are not limited to, the following:(1,2)

- Patients with one or more congenital anomalies
- Patients with developmental delay or intellectual disability with age of onset prior to 18 years
- Patients with a phenotype or family history that strongly suggests an underlying genetic cause, yet genetic tests for that phenotype have failed to arrive at a diagnosis (diagnostic odyssey)
- Patients with a phenotype or family history that strongly suggests an underlying genetic cause, but the phenotype does not fit with one specific disorder (numerous individual genetic tests would be required for evaluation)
- Patients with a suspected genetic disorder that has numerous underlying genetic causes, making analysis of numerous genes simultaneously a more practical approach than single-gene testing (condition is genetically heterogeneous)
- Patients with a suspected genetic disorder for which specific molecular genetic testing is not yet available
- Patients with an atypical presentation of a genetic disorder

It is highly recommended that specimens are also submitted from the patient's biological mother and biological father, which are used for comparison purposes (trio analysis). However, testing for the patient only (singleton), the patient and one first-degree relative (duo), and the patient and two first-degree relatives (nontraditional trios) will also be accepted if the patient's biological mother and biological father are not available for testing. Testing typically includes up to two family member comparators. Contact the laboratory for approval to send the patient and three first-degree relatives (quad).

Based upon published reports, a diagnosis is identified by whole genome sequencing (WGS) in up to 44% of cases.(3,4) In comparison to whole exome sequencing (WES), WGS has advantages as it is more comprehensive, detects additional variant types, and includes coverage outside of the exons.(5) WGS has been shown to identify additional diagnoses in patients who previously had WES with negative or inconclusive results.(3,6-8)

## Reference Values

An interpretive report will be provided.

## Interpretation

Variants of interest are evaluated according to American College of Medical Genetics and Genomics recommendations.(9,10) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Variants are reported in one of the following categories:

- Likely Causative: Variants with a high degree of suspicion for causing the patient's reported clinical features
- Possibly Relevant: Variants that may be related to the patient's clinical features or variants in genes of uncertain significance
- Secondary Findings: Medically actionable variants unrelated to the indication for testing (see below for additional information)

It is possible that a variant may not be recognized as the underlying cause of disease due to incomplete scientific knowledge about the function of all genes in the human genome and/or the impact of variants in those genes.

Secondary Findings:

Patients are evaluated for medically actionable secondary findings and these findings are reported in accordance with the American College of Medical Genetics and Genomics recommendations.<sup>(11)</sup> Variants in these genes will not be evaluated or reported if the patient opts out of this evaluation unless they overlap with the patient's reported clinical features.

The presence of a secondary finding in family member comparator samples is stated on the patient's (proband's) report unless family members opt out of secondary findings. If the patient (proband) opts out, secondary findings will not be evaluated or reported in any family member comparators. Secondary findings that are present in family member comparators but absent from the patient (proband) are not evaluated or reported.

The absence of a reportable secondary finding does not guarantee that there are no disease-causing or likely disease-causing variants in these genes, as review is limited to known or highly suspected pathogenic findings, and not all regions of these genes are adequately evaluated by this technology.

#### Reanalysis and Raw Data Requests:

It is not guaranteed that patient data will be stored indefinitely. Requests for reanalysis or release of raw data may not be possible, and a new whole genome sequencing order may be required if the original patient data is no longer available or no longer compatible with current bioinformatics processes or analysis tools.

Healthcare providers may contact the laboratory at 800-533-1710 to request reanalysis of the patient's genome due to new patient clinical features, advances in genetic knowledge, or changes in testing methodology. A charge may apply for reanalysis.

Requests for the raw data obtained from whole genome sequencing should be directed to the laboratory. A separate fee may apply. Raw data will be released for individuals who complete a Mayo Clinic release of information form. If raw data for family member comparators is requested, it will only be released with an accompanying request for the proband's raw data. Contact the laboratory for instructions on completing the release of information form. The laboratory is not responsible for providing software or other tools needed to visualize, filter, or interpret this data.

#### 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.

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:

Whole genome sequencing may not detect all types of genomic variants; therefore, false-negative results are possible. There may be regions of genes that cannot be effectively evaluated by whole genome sequencing as a result of technical limitations of the assay, including variable depth of coverage, regions of homology, and repetitive sequences. 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. In addition, in rare cases false-positive results may occur; however, false-positive events should be exceedingly rare as confirmation of reportable variants will be



performed by alternate methodologies based on internal laboratory criteria.

#### Single Nucleotide Variants:

Genome-wide sensitivity for single nucleotide variants (SNV) is greater than 98% and in noncomplex regions sensitivity is greater than 99.9%.

#### Deletions and Insertions (less than 1000 base pairs):

This test is validated to detect greater than 99% of deletions and insertions (delins) up to 50 base pairs (bp) for noncomplex regions. Performance in complex regions is slightly reduced with sensitivities of 92% to 99% depending on the size of the delins event. Although detected by this assay, performance for larger delins (51-999 bp) is not comprehensively established.

#### Copy Number Variants (greater than or equal to 1000 base pairs):

Genome-wide sensitivity for copy number variants (CNV) detectable by chromosomal microarray is greater than 99.9% as established by a comprehensive comparison with clinically validated non-mosaic CNV detected by chromosomal microarray.

#### Additional Variant Classes:

A variety of additional variant classes can be detected by this test, including mitochondrial variants, repeat expansions, select spinal muscular atrophy (SMA)-associated variants, balanced structural rearrangements, and mosaic variants of all classes. A limited validation of each of these additional variant classes was conducted before inclusion in this assay; however, comprehensive assessment of sensitivity and false-negative rate has not been established. These variants will be evaluated per laboratory protocol, and findings of clinical relevance will be reported following confirmation with validated laboratory methods. Importantly, the sensitivity and performance for these variant classes is not expected to meet that of current gold-standard testing methodologies; therefore, additional testing may be indicated if there is clinical suspicion for a disorder involving these variant classes. Specific technical limitations for each class are described below.

#### Mitochondrial Genome Variants:

This assay can detect mitochondrial genome SNV and small delins with heteroplasmy levels above 5%; however, comprehensive sensitivity/false-negative rate is not established. Detection of large deletion and duplication events involving the mitochondrial genome is not available with the current analysis. Any clinically relevant and reportable mitochondrial variants detected will be confirmed with standard validated methods prior to reporting.

#### Repeat Expansion Variants:

Select short tandem repeats (STR; also known as repeat expansions) in pathogenic ranges can be detected with this assay; however, comprehensive sensitivity/false negative rate is not established. STR loci included in this assay are: *C9orf72*, *CSTB*, *ATN1*, *FXN*, *FMR1*, *HTT*, *AR*, *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7*. Only loci overlapping the patient's (proband's) clinical features will be evaluated and reported. All repeat expansions meeting laboratory reporting criteria will be confirmed and further characterized by standard validated methods prior to reporting.

#### SMA Variants:

*SMN1* copy number and *SMN2* copy number are determined. This assay also ascertains whether the g.27134T>G alteration is present or absent in patients found to have 2 copies of *SMN1*. All variants will be confirmed by validated



laboratory methods before reporting.

#### Balanced Structural Rearrangements:

This assay does not involve comprehensive evaluation of balanced structural rearrangements (eg, translocations and inversions). However, select genomic regions may be evaluated and balanced events reported when there is a directed clinical focus (eg, known family history or specific locus of high clinical suspicion communicated to the laboratory). Comprehensive sensitivity/false-negative rate is not established. All reported rearrangements will be confirmed by validated laboratory methods before reporting (additional specimen may be required for further characterization).

#### Mosaicism:

This assay is not designed to detect mosaicism or to differentiate between somatic and germline variants. Mosaic variants may be detected; however, comprehensive limits of detection for mosaic events are not established. All mosaic variants meeting laboratory reporting criteria will be confirmed by validated laboratory methods before reporting.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent 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 providers 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 (ACMG) recommendations as a guideline.(9,10) 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.

Rarely, incidental or secondary findings outside of the genes recommended by the ACMG may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

#### Data Sharing:

Deidentified variant information may be shared in public genetic databases, such ClinVar and Matchmaker Exchange.

A genetic consultation is recommended for patients undergoing this test, both prior to testing and after results are available.

#### Clinical Reference

1. Manickam K, McClain MR, Demmer LA, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and

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- Genomes (ACMG). *Genet Med.* 2021;23(11):2029-2037. doi:10.1038/s41436-021-01242-6
2. ACMG Board of Directors. Points to consider for informed consent for genome/exome sequencing. *Genet Med.* 2013;15(9):748-749. doi:10.1038/gim.2013.94
3. Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med.* 2018;20(4):435-443. doi:10.1038/gim.2017.119
4. Lee HF, Chi CS, Tsai CR. Diagnostic yield and treatment impact of whole-genome sequencing in paediatric neurological disorders. *Dev Med Child Neurol.* 2021;63(8):934-938. doi:10.1111/dmcn.14722
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6. Splinter K, Adams DR, Bacino CA, et al. Effect of genetic diagnosis on patients with previously undiagnosed disease. *N Engl J Med.* 2018;379(22):2131-2139. doi:10.1056/NEJMoa1714458
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8. Palmer EE, Sachdev R, Macintosh R, et al. Diagnostic yield of whole genome sequencing after nondiagnostic exome sequencing or gene panel in developmental and epileptic encephalopathies. *Neurology.* 2021;96(13):e1770-e1782. doi:10.1212/WNL.00000000000011655
9. 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):405-24. doi:10.1038/gim.2015.30
10. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) [published correction appears in *Genet Med.* 2021 Nov;23(11):2230]. *Genet Med.* 2020;22(2):245-257. doi:10.1038/s41436-019-0686-8
11. Miller DT, Lee K, Gordon AS, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2021;23(8):1391-1398. doi:10.1038/s41436-021-01171-4

## Performance

### Method Description

Next-generation sequencing is performed on DNA extracted from the patient (proband) and all submitted comparator samples (if applicable) to test for the presence of variants. The human genome reference GRCh38/hg38 build is used for sequence read alignment. Variants are called using an optimized bioinformatics package. The average genomic coverage is at or above a read depth of 32X. Sensitivity is estimated to be above 99% for single nucleotide variants, and above 94% for deletion-insertions (delins) less than 50 base pairs (bp). This assay also detects greater than 99% copy number variants (deletions/duplications) at least 1000 bp in size. Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.

There may be regions of genes that cannot be effectively evaluated by sequencing as a result of technical limitations of the assay, including regions of homology, variable depth of coverage, and repetitive sequences. (Unpublished Mayo

method)

## PDF Report

Supplemental

## Day(s) Performed

Varies

## Report Available

84 days

## Specimen Retention Time

Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Cord blood, saliva, cultured fibroblasts, skin biopsy: 1 month

## 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. It has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information

81425-Patient only

81425, 81426-Patient and one family member comparator sample (duo) (as appropriate)

81425, 81426 x 2-Patient and two family member comparator samples (trio or non-traditional trio) (as appropriate)

81425, 81426 x 3-Patient and three family member comparator samples (quad) (as appropriate)

88233-Tissue culture, skin, solid tissue biopsy (if appropriate)

88240-Cryopreservation (if appropriate)

### LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WGSDX	Whole Genome Sequencing	86206-0

Result ID	Test Result Name	Result LOINC® Value
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614364	Interpretation	69047-9
614464	Specimen	31208-2
614317	Source	31208-2
614473	Released By	18771-6