MAYO CLINIC LABORATORIES

Test Definition: BTKSG

Bruton Tyrosine Kinase, BTK Full Gene Analysis, Varies

Overview

Useful For

Confirming a diagnosis of X-linked agammaglobulinemia in patients with a history of recurrent sinopulmonary infections, profound hypogammaglobulinemia, and less than 1% peripheral B cells, with or without abnormal Bruton tyrosine kinase (BTK) protein expression by flow cytometry

Evaluating for the presence of *BTK* variants in family members of affected individuals, including those who do not demonstrate carrier phenotype by BTK flow cytometry

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
CULFB	Fibroblast Culture for Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *BTK* gene associated with X-linked agammaglobulinemia.

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

Testing Algorithm

Skin biopsy or cultured fibroblast specimens:

For skin biopsy or cultured fibroblast specimens, a fibroblast culture will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Cord blood:

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

Special Instructions

- Informed Consent for Genetic Testing

- Bruton Tyrosine Kinase (BTK) Genotype Patient Information
- Informed Consent for Genetic Testing (Spanish)

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

Additional Testing Requirements
To confirm a diagnosis of X-linked agammaglobulinemia in male patients, the preferred approach is to order this test concurrently with BTK / Bruton Tyrosine Kinase, Protein Expression, Flow Cytometry, Blood.

Necessary Information
Bruton Tyrosine Kinase (BTK) Gene Sequencing Patient Information form (T620) is highly recommended. Testing may proceed without the patient information. However, it aids in providing a more thorough interpretation. 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: 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: 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. Due to lower quantity/quality of DNA yielded from blood spots, 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.
2. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
3. For collection instructions, see Blood Spot Collection Instructions
4. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
5. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

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: 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: Cultured fibroblasts
Source: Skin or tissue
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a biopsy. Cultured cells from a prenatal specimen will not be accepted.
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: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2mL 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.

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. Bruton Tyrosine Kinase (BTK) Gene Sequencing Patient Information form (T620)

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

X-linked agammaglobulinemia (XLA) is a humoral primary immunodeficiency affecting male patients in approximately 1 in 200,000 live births. XLA is caused by variants in the Bruton tyrosine kinase gene (*BTK*), which results in a profound block in B-cell development within the bone marrow and a significant reduction, or complete absence, of mature B cells in peripheral blood.(1,2)

Approximately 85% of male patients with defects in early B-cell development have XLA. Due to the lack of mature B cells, XLA patients have markedly reduced levels of all major classes of immunoglobulins in the serum and are, therefore,

susceptible to severe and recurrent bacterial infections.(2) Pneumonia, otitis media, enteritis, and recurrent sinopulmonary infections are among the key diagnostic clinical characteristics of the disease. The spectrum of infectious complications also includes enteroviral meningitis, septic arthritis, cellulitis, and empyema, among others. XLA typically manifests in male infants.(2) However, other patients present with milder phenotypes, resulting in diagnosis later in childhood or in adulthood. Delayed diagnoses can be partly explained by the variable severity of XLA, even within families in which the same variant is present. X-inactivation of this gene is not typical, and XLA in female patients has rarely been reported.(3) Therefore, female patients with clinical features that are identical to XLA should be first evaluated for autosomal recessive agammaglobulinemia and for XLA if their biological father is affected with the disease.

A diagnosis of XLA should be suspected in male patients with early-onset bacterial infections, marked reduction in all classes of serum immunoglobulins, and absent B cells (CD19+ cells). The decrease in numbers of peripheral B cells is a key feature, although this can also be seen in a small subset of patients with common variable immunodeficiency. Conversely, some *BTK* variants can preserve small numbers of circulating B cells and, therefore, all 3 of the criteria mentioned above need to be evaluated.(2)

The preferred approach for confirming a diagnosis of XLA in male patients and identifying female carriers requires testing for the BTK protein expression on B cells by flow cytometry and genetic testing for a *BTK* variant. Patients can be screened for the presence of BTK protein by flow cytometry (BTK / Bruton Tyrosine Kinase, Protein Expression, Flow Cytometry, Blood); however, normal results by flow cytometry do not rule out the presence of a *BTK* variant with normal protein expression but aberrant protein function. The diagnosis is confirmed only in those individuals with appropriate clinical history who have a disease-causing variant identified within *BTK* by gene sequencing or who have male family members with hypogammaglobulinemia with absent or low B cells.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(4) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Bruton tyrosine kinase (BTK) protein and genetic tests are not meant for patients with hematological neoplasias on kinase inhibitor therapy, including, but not restricted to, the selective BTK inhibitor, Ibrutinib. This test is meant for the assessment of patients with a suspected monogenic primary immunodeficiency, X-linked agammaglobulinemia, caused by germline variants in the *BTK* gene.

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 genes 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 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.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic 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-leukocyte reduced 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.(4) 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. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. Tsukada S, Saffran DC, Rawlings DJ, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993;72(2):279-290
2. El-Sayed ZA, Abramova I, Aldave JC, et al. X-linked agammaglobulinemia (XLA):Phenotype, diagnosis, and therapeutic challenges around the world. World Allergy Organ J. 2019;12(3):100018.doi:10.1016/j.waojou.2019.100018
3. Takada H, Kanegane H, Nomura A, et al. Female agammaglobulinemia due to the Bruton tyrosine kinase deficiency caused by extremely skewed X-chromosome inactivation. Blood;103(1):185-187
4. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee: 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
5. Hernandez-Trujillo V, Zhou C, Scalchunes C, et al. A registry study of 240 patients with X-linked agammaglobulinemia living in the USA. J Clin Immunol. 2023;43(6):1468-1477
6. Lopez-Granados E, Perez de Diego R, Ferreira Cerdan A, Fontan Casariego G, Garcia Rodríguez MC. A genotype-phenotype correlation study in a group of 54 patients with X-linked agammaglobulinemia. J Allergy Clin Immunol. 2005;116(3):690-697. doi:10.1016/j.jaci.2005.04.043

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the *BTK* 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 30X. Sensitivity is estimated at above 99% for single nucleotide

variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), and 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 *BTK* gene.

There may be regions of *BTK* 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)

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

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.

Gene symbol	Reference transcript	Additional evaluations	Technical limitations
BTK	NM_000061.2	c.-193A>G c.142-205A>G c.240+108T>G c.240+109C>A c.895-11C>A c.1102+2_1102+12del c.1177+26_1177+27insGGTAGAAAAAA c.1567-23A>C c.1567-23A>G c.1567-12_1567-9del	-

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
21 to 28 days

Specimen Retention Time
Whole blood: 25 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 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

81406

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

88240- Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BTKSG	BTK Gene, Full Gene Analysis	94241-7

Result ID	Test Result Name	Result LOINC® Value
619761	Test Description	62364-5
619762	Specimen	31208-2
619763	Source	31208-2
619764	Result Summary	50397-9
619765	Result	82939-0
619766	Interpretation	69047-9
619767	Additional Results	82939-0
619768	Resources	99622-3
619769	Additional Information	48767-8
619770	Method	85069-3
619771	Genes Analyzed	82939-0
619772	Disclaimer	62364-5
619773	Released By	18771-6