
Overview

Useful For

Preliminary screening for X-linked agammaglobulinemia, primarily in male patients (<65 years of age) or female carriers (child-bearing age: <45 years)

Genetics Test Information

The Bruton tyrosine kinase (*BTK*) gene is present on the long arm of the X-chromosome and encodes the intracellular signaling protein BTK critical for B-lymphocyte development and function. Loss of function variants in this gene cause X-linked agammaglobulinemia in male patients.

Method Name

Flow Cytometry

NY State Available

No

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

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 only meant for the assessment of patients with a suspected monogenic primary immunodeficiency, X-linked agammaglobulinemia, caused by germline variants in the Bruton tyrosine kinase gene.

The preferred test for confirming a diagnosis of X-linked agammaglobulinemia in male patients and identifying female carriers is BTKFP / Bruton Tyrosine Kinase (*BTK*) Genotype and Protein Analysis, Full Gene Sequence and Flow Cytometry, Blood.

In families where a *BTK* variant has already been identified, order FMTT / Familial Mutation, Targeted Testing, Varies.

Shipping Instructions

Specimens are required to be received in the laboratory weekdays and by 4 p.m. on Friday. Collect and package specimen as close to shipping time as possible.

It is recommended that specimens arrive within 24 hours of collection.

Samples arriving on the weekend and observed holidays may be canceled.

Necessary Information

Ordering physician's name and phone number are required.

Specimen Required

Container/Tube: Lavender top (EDTA)

Specimen Volume: 4 mL

Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**

Specimen Minimum Volume

2 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Ambient	48 hours	PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

The differential diagnosis for patients with primary hypogammaglobulinemia of unclear etiology (after other secondary causes of hypogammaglobulinemia have been ruled out) includes common variable immunodeficiency (CVID) and X-linked agammaglobulinemia (XLA). CVID is the most common diagnosis of humoral immunodeficiency, particularly in adults but also in children over 4 years of age. However, men with XLA may be misdiagnosed with CVID. XLA is an independent humoral immunodeficiency and should not be regarded as a subset of CVID.

The *BTK* gene is present on the long arm of the X-chromosome and encodes for a cytoplasmic tyrosine kinase with 5 distinct structural domains. While *BTK* gene sequencing is the gold standard for definitively identifying variants and confirming a diagnosis of XLA, it is labor intensive and expensive, and it may result in a variant of uncertain significance (VUS). Flow cytometry is a screening test for XLA and should be included in the evaluation of patients with possible CVID, particularly in male patients with less than 1% B cells. Bruton tyrosine kinase (BTK) is an intracellular protein, and absence of the BTK protein by flow cytometry provides a strong rationale for performing a *BTK* gene-sequencing test. However, 20% to 30% of patients with XLA may have intact or truncated BTK protein with abnormal function; therefore, genetic analysis remains the more definitive test for diagnosing XLA (besides other clinical and immunological parameters).

XLA is a prototypical humoral immunodeficiency caused by variants in the *BTK* gene, which encodes BTK, a hematopoietic-specific tyrosine kinase. XLA is characterized by normal, reduced, or absent BTK expression in monocytes and platelets, a significant reduction or absence of circulating B cells in blood, and profound hypogammaglobulinemia of all isotypes (IgG, IgA, IgM, and IgE). The clinical presentation includes early onset of recurrent bacterial infections and absent lymph nodes and tonsils. BTK plays a critical role in B-cell differentiation. The defect in BTK may be "leaky" in some patients (ie, a consequence of variants in the gene that result in a milder clinical and laboratory phenotype), such that these patients may have some levels of IgG and/or IgM and a small number of B cells in blood.(1)

The vast majority of patients with XLA are diagnosed in childhood (median age of diagnosis in patients with sporadic XLA is 26 months), although some patients are recognized in early adulthood or later in life. The diagnosis of XLA in both children and adults indicates that the disorder demonstrates considerable clinical phenotypic heterogeneity, depending on the position of the variants within the gene. Female patients are typically carriers and asymptomatic. Testing in women should be limited to those in their child-bearing years (<45 years). Carrier testing ideally should be confirmed by genetic testing since it is possible to have a normal flow cytometry test for protein expression in the presence of heterozygous (carrier) *BTK* gene variants.

Flow cytometry is a preliminary screening test for XLA. It is important to keep in mind that this flow cytometry test is only a screening tool and approximately 20% to 30% of patients who have a variant within the *BTK* gene have normal protein expression (again related to the position of the variant in the gene and the antibody used for flow cytometric analysis). Therefore, in addition to clinical correlation, genetic testing is recommended to confirm a diagnosis of XLA. Furthermore, it is helpful to correlate gene and protein data with clinical history (genotype-phenotype correlation) in making a final diagnosis of XLA. Consequently, the preferred test for XLA is BTKFP / Bruton Tyrosine Kinase (*BTK*) Genotype and Protein Analysis, Full Gene Sequence and Flow Cytometry, Blood, which includes both flow cytometry and gene sequencing to confirm the presence of a *BTK* variant. If a familial variant has already been identified, then FMTT / Familial Mutation, Targeted Testing, Varies should be ordered.

Reference Values

[Present](#)

Bruton tyrosine kinase expression will be reported as present, absent, partial deficiency, or mosaic (carrier).

Interpretation

Results are reported as Bruton tyrosine kinase (BTK) protein expression present (normal) or absent (abnormal) in monocytes and B cells if present. Additionally, mosaic BTK expression (indicative of a carrier) and reduced BTK expression (consistent with partial BTK protein deficiency) are reported when present and correlated with a healthy experimental control.

BTK genotyping (BTKS / Bruton Tyrosine Kinase [*BTK*] Genotype, Full Gene Sequence, Blood or FMTT / Familial Mutation, Targeted Testing, Varies) should be performed in the following situations:

- To confirm any abnormal flow cytometry result
- In the rare patient with the clinical features of X-linked agammaglobulinemia, but normal BTK protein expression
- In mothers of patients who do not show the classic carrier pattern of bimodal protein expression (to determine if there is maternal germinal mosaicism or skewed altered X-chromosome inactivation), or there is dominant expression of the normal protein in the presence of one copy of a genetic variant.

Cautions

This test is typically not indicated women beyond child-bearing age or in men greater than 65 years of age, unless there is a strong clinical and family history, and the patient has not received a formal diagnosis and may or may not be on replacement immunoglobulin therapy. For questions about appropriate test selection, call 800-533-1710.

The flow cytometry screening assay is likely to detect the majority of patients with X-linked agammaglobulinemia (XLA) and completely or partially deficient Bruton tyrosine kinase (BTK) protein expression. However, approximately 20% to 30% of male patients may have normal BTK protein expression with aberrant function that can only be detected by *BTK* gene sequencing. The ability to identify female carriers by the flow cytometry assay is largely dependent on the BTK-specific antibodies used for flow detection. In general, genetic testing is preferable and more definitive to flow cytometry for identification of female carriers.

It is also important to note that there are patients with XLA whose mothers have normal BTK protein expression by flow cytometry and normal *BTK* genotyping, and the genetic variant in the patient is a result of *de novo* variants in the germline *BTK* gene.(1) In the same study, it has been shown that there can be female carriers who have normal BTK protein expression but who are genetically heterozygous and do not show abnormal protein expression due to extreme skewed inactivation of the altered X chromosome.

Clinical Reference

1. Kanegane H, Futatani T, Wang Y, et al: Clinical and mutational characteristics of X-linked agammaglobulinemia and its carrier identified by flow cytometric assessment combined with genetic analysis. *J Allergy Clin Immunol*. 2001 Dec;108(6):1012-1020. doi: 10.1067/mai.2001.120133
2. Kanegane H, Tsukada S, Iwata T, et al: Detection of Bruton's tyrosine kinase mutations in hypogammaglobulinemic males registered as common variable immunodeficiency (CVID) in the Japanese Immunodeficiency Registry. *Clin Exp Immunol*. 2000 Jun;120(3):512-517. doi: 10.1046/j.1365-2249.2000.01244.x
3. Stewart DM, Tian L, Nelson DL: A case of X-linked agammaglobulinemia diagnosed in adulthood. *Clin Immunol*. 2001 Apr;99(1):94-99. doi: 10.1006/clim.2001.5024
4. Futatani T, Miyawaki T, Tsukada S, et al: Deficient expression of Bruton's tyrosine kinase in monocytes from X-linked agammaglobulinemia as evaluated by a flow cytometric analysis and its clinical application to carrier detection. *Blood*. 1998 Jan 15;91(2):595-602
5. Kraft MT, Pyle R, Dong X, et al: Identification of 22 novel BTK gene variants in B cell deficiency with hypogammaglobulinemia. *Clin Immunol*. 2021 Aug;229:108788. doi: 10.1016/j.clim.2021.108788
6. Chear CT, Ripen AM, Mohamed SAS, Dhaliwal JS: A novel BTK gene mutation creates a de-novo splice site in an X-linked agammaglobulinemia patient. *Gene*. 2015 Apr 15;560(2):245-248. doi: 10.1016/j.gene.2015.02.019

Performance

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 4 days

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 using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

88184

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BTK	Btk Protein Flow, B	75708-8

Result ID	Test Result Name	Result LOINC® Value
89011	Btk Protein Flow, B	75708-8