# Test Definition: BTK

Bruton Tyrosine Kinase, Protein Expression, Flow Cytometry, Blood

## Overview

### Useful For

Preliminary screening for X-linked agammaglobulinemia, primarily in male patients (<65 years) 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 BTKSG / Bruton Tyrosine Kinase, *BTK* Full Gene Analysis, Varies.

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

### Shipping Instructions

**Testing is not performed on Saturday, Sunday, or observed holidays. Only collect and ship specimen for arrival on days when testing is performed.**

**Specimens received on days when testing is not performed or after 5 p.m. Central on Friday may be canceled if specimen is outside of stability when testing is next performed.**

Collect and package specimen as close to shipping time as possible. It is recommended that specimens arrive within 24 hours of collection.

### Necessary Information

**Ordering healthcare professional 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. However, men with XLA may be misdiagnosed with CVID.

The *BTK* gene is present on the long arm of the X-chromosome and encodes for a cytoplasmic tyrosine kinase. While *BTK* gene sequencing is the gold standard for definitively identifying variants and confirming a diagnosis of XLA, it is labor intensive and it may result in a variant of uncertain significance. 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).

X-linked agammaglobulinemia 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)

Most 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 BTKSG / Bruton Tyrosine Kinase, *BTK* Full Gene Analysis, Varies. If a familial variant has already been identified, then FMTT / Familial Variant, Targeted Testing, Varies should be ordered.

### Reference Values

Present

### 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 (BTKSG / Bruton Tyrosine Kinase, BTK Full Gene Analysis, Varies or FMTT / Familial Variant, 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 for women beyond child-bearing age or in men greater than 65 years, 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;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;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;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;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;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;560(2):245-248. doi:10.1016/j.gene.2015.02.019

## Performance

### Method Description

The Bruton tyrosine kinase (BTK) protein expression screening assay is performed on a whole blood sample. The cells in the blood are stained with antihuman CD20 (B cells) and CD14 (monocytes) antibodies followed by red blood cell lysis (using a premade Lysis buffer), cell fixation, and permeabilization to prepare the cell membrane for the anti-human BTK antibody. After the permeabilization step, the cells are stained for intracellular BTK using antihuman BTK-fluorescent pre-conjugated antibody. After the staining and wash process, the cells are fixed and analyzed by multiparametric flow cytometry.(Unpublished Mayo method)

# Test Definition: BTK

Bruton Tyrosine Kinase, Protein Expression, Flow Cytometry, Blood

### PDF Report
No

### Day(s) Performed
Monday through Friday

### Report Available
3 to 4 days

### Specimen Retention Time
4 days

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

## 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 |