Test Definition: BTKFP

BTK Full-Gene Panel, B

Overview

Useful For

Preferred test for confirming a diagnosis of X-linked agammaglobulinemia (XLA) in males with a history of recurrent sinopulmonary infections, profound hypogammaglobulinemia, and below 1% peripheral B cells

In females, this is the most useful test for identifying carriers of XLA.

By including protein and gene analysis, this test provides a comprehensive assessment and enables appropriate genotype-phenotype correlations.

Testing Algorithm

Special Instructions

- Bruton Tyrosine Kinase (BTK) Genotype Patient Information

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
BTKSP	BTK, Full Gene Sequence	Yes, (order BTKS)	Yes
BTKSQ	BTK, Full Gene Sequencing	No	Yes
BTK	Btk Protein Flow, B	Yes	Yes

Method Name

BTKSP: Polymerase Chain Reaction (PCR) Followed by DNA Sequence Analysis

BTK: Flow Cytometry

NY State Available

No

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

New York Clients: The BTK / Bruton Tyrosine Kinase (Btk), Protein Expression, Flow Cytometry, Blood portion of this test is not New York State approved. Order BTKS / Bruton Tyrosine Kinase (*BTK*) Genotype, Full Gene Sequence, Blood.

Testing for familial variants/known mutations is available; see 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. Draw and package specimen as close to shipping time as possible.

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

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

Necessary Information

Ordering physician's name, phone number, and patient information are required.

Specimen Required

Two separate EDTA specimens and the patient information sheet are required.

Specimen Type: Blood for BTKSP / Bruton Tyrosine Kinase (*BTK*) Genotype, Full Gene Sequence

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions:

1. Send specimen in original tube.

2. Label as BTKSP.

Specimen Stability Information: Refrigerated (preferred)/Ambient

Specimen Type: Blood for BTK / Bruton Tyrosine Kinase (Btk), Protein Expression, Flow Cytometry, Blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 4 mL

Collection Instructions:

1. Send specimen in original tube. **Do not aliquot.**

2. **Ship at ambient temperature only.**

3. Label as BTK.

Specimen Stability Information: Ambient 72 hours

Additional Information: For flow cytometry serial monitoring, we recommend that specimen draws be performed at the same time of day.

Forms

Bruton Tyrosine Kinase (*BTK*) Genotype Patient Information **(T620) is required.** see Special Instructions

Reject Due To

No specimen should be rejected.

Specimen Minimum Volume

BTKSP: 0.35 mL

BTK: 2 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Varies (preferred)		

Clinical & Interpretive

Clinical Information

X-linked agammaglobulinemia (XLA) is a humoral primary immunodeficiency affecting males in approximately 1 in 200,000 live births. XLA is caused by variants in the Bruton tyrosine kinase gene (*BTK*),(1) 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.(2) Approximately 85% of male patients with defects in early B-cell development have XLA.(3) 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. Pneumonia, otitis media, enteritis, and recurrent sinopulmonary infections are among the key clinical diagnostic characteristics of the disease. The spectrum of infectious complications also includes enteroviral meningitis, septic arthritis, cellulitis, and empyema, among others. The disease typically manifests in male children younger than 1 year of age.

BTK, the only gene associated with XLA, maps to the X-chromosome at Xq21.3-Xq22 and consists of 19 exons spanning 37.5 kb genomic DNA.(4) *BTK* encodes a nonreceptor tyrosine kinase of the Btk/Tec family. The Bruton tyrosine kinase (Btk) protein consists of 5 structural domains (PH, TH, SH3, SH2, and TK). Variants causing XLA have been found in all domains of the *BTK* gene, as well as noncoding regions of the gene. Missense variants account for 40% of all variants, while nonsense variants account for 17%, deletions 20%, insertions 7%, and splice-site variants 16%. Over 600 unique variants in the *BTK* gene have been detected by full gene sequencing and are listed in BTKbase, a database for *BTK* variants (http://bioinf.uta.fi/BTKbase).(5) Genotype-phenotype correlations have not been completely defined for *BTK*, but it is clear that nonsense variants are overrepresented 4-fold compared to substitutions, which indicates that the latter may be tolerated without causing a phenotype. The type and location of the variant in the gene clearly affects the severity of the clinical phenotype. Some variants manifest within the first year or 2 of life, enabling an early diagnosis. Others present with milder phenotypes, resulting in diagnosis later in childhood or in adulthood.(5) Delayed diagnoses can be partly explained by the variable severity of XLA, even within families in which the same variant is present. While the disease is considered fully penetrant, the clinical phenotype can vary considerably depending on the nature of the specific *BTK* variant.(5) Lyonization of this gene is not typical and only 1 case of XLA in a female has been reported so far due to skewed lyonization in a carrier female. Therefore, females with clinical features that are identical to XLA should be evaluated for autosomal recessive agammaglobulinemia when deemed clinically appropriate(6) and for XLA, if a male parent is affected with the disease.

A flow cytometry test for intracellular Btk in monocytes using an anti-Btk monoclonal antibody was developed by Futatani et al, which was used to evaluate both XLA patients and carriers.(7) In this study, 41 unrelated XLA families were studied and deficient Btk protein expression was seen in 40 of these 41 patients, with complete Btk deficiency in 35 patients and partial Btk deficiency in 5 patients. One patient had a normal level of Btk protein expression. The 6 patients with partial or normal Btk expression had missense *BTK* variants. Additionally, the flow cytometry assay detected carrier status in the mothers of 35 of the 41 patients (approximately 85%). In the 6 patients where the Btk expression was normal in the mothers of XLA patients, it was noted that all these patients were sporadic cases without previous family history of the disease.(7)

It appears, therefore, that most *BTK* variants result in deficient expression of Btk protein, which can be detected by flow cytometry in monocytes.(7,8) Also, the mosaic expression of Btk protein in the monocytes by flow cytometry is potentially useful in the diagnosis of female carriers.(8) The flow cytometry test therefore provides a convenient screening tool for the diagnosis of XLA with confirmation of the diagnosis by *BTK* genotyping.(7,8) In the rare patient with the clinical features of XLA but normal Btk protein expression, *BTK* genotyping must be performed to determine the presence of a variant.

A diagnosis of XLA should be suspected in males with 1) early-onset bacterial infections, 2) marked reduction in all classes of serum immunoglobulins, and 3) absent B cells (CD19+ cells). The decrease in numbers of peripheral B cells is a key feature, though this also can be seen in a small subset of patients with common variable immunodeficiency (CVID). As well, some *BTK* variants can preserve small numbers of circulating B cells and, therefore, all the 3 criteria mentioned above need to be evaluated. Patients should be assessed for the presence of Btk protein by flow cytometry, although

normal results by flow cytometry do not rule out the presence of a *BTK* variant with aberrant protein function (despite normal protein expression). The diagnosis is established or confirmed only in those individuals who have a variant identified in the *BTK* gene by gene sequencing or who have male family members with hypogammaglobulinemia with absent or low B cells. Appropriate clinical history is required with or without abnormal Btk protein results by flow cytometry.

It was shown that there are XLA patients with mothers who have normal Btk protein expression by flow cytometry and normal *BTK* genotyping and that the variant in the patient is a result of *de novo* variants in the maternal germline. In the same study, it was shown that there can be female carriers who have normal Btk protein expression but are genetically heterozygous and they do not show abnormal protein expression due to extreme skewed inactivation of the mutant X-chromosome.(6) Also, the presence of 1 copy of the normal *BTK* gene and associated normal Btk protein can stabilize mutant protein abrogating the typical bimodal pattern of protein expression seen in female carriers. Therefore, female carrier status can only conclusively be determined by genetic testing, especially if the Btk protein flow test is normal.

It is important to keep in mind that the mere presence of *BTK* gene variants does not necessarily correlate with a diagnosis of XLA unless the appropriate clinical and immunological features are present.(9,10)

Reference Values

BTKSP: An interpretive report will be provided.

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

Interpretation

A patient-specific interpretive report is provided.

Cautions

Some X-linked agammaglobulinemia (XLA) patients may have higher B cells than 1% but still be below the reference value for age and, also, immunoglobulins may be low or normal, initially. If family history, age, clinical and immunological history raise the suspicion for XLA, this test can be ordered.

Patients who have received a heterologous blood transfusion within the preceding 6 weeks, or who have received an allogeneic blood or marrow transplant, can have inaccurate genetic test results due to presence of donor DNA.

Rare variants could potentially lead to false-negative or false-positive results. If results obtained do not match clinical findings, additional testing should be considered. Any error in the diagnosis or in the pedigree provided to the laboratory

could lead to an erroneous interpretation of results.

This method will not detect variants that occur in intronic (other than exon-intron boundaries) and regulatory regions of the gene or large rearrangement-type variants (which could cause a false-negative result).

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, Ibrutonib. 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.

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;72:279-290

2. Noordzij JG, de Bruin-Versteeg S, Comans-Bitter WM, et al: Composition of precursor B-cell compartment in bone marrow from patients with X-linked agammaglobulinemia compared with health children. Pediatr Res 2002;2:159-168

3. Conley ME, Broides A, Hernandez-Trujillo V, et al: Genetic analysis of patients with defects in early B-cell development. Immunol Rev 2005;203:216-234

4. Lindvall JM, Blomberg KEM, Vargas L, et al: Bruton's tyrosine kinase: cell biology, sequence conservation, mutation spectrum, siRNA modifications, and expression profiling. Immunol Rev 2005;203:200-215

5. Valiaho J, Smith CI, Vihinen M: BTKbase: The mutation database for X-linked agammaglobulinemia. Hum Mutat 2006;27:1209-1217

6. Takada H, Kanegane H, Nomura A, et al: Female agammaglobulinemia due to the Bruton's tyrosine kinase deficiency caused by extremely skewed X-chromosome inactivation. Blood 2004;103:185-187

7. 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

8. 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;108:1012-1020

9. Graziani S, Di Matteo G, Benini L, et al: Identification of a *BTK* mutation in a dysgammaglobulinemia patient with reduced B cells: XLA or not? Clin Immunol 2008;128:322-328

10. Fleisher TA, Notarangelo LD: What does it take to call it a pathogenic mutation? Clin Immunol 2008;128:285-286

Performance

Method Description

Genomic DNA is first extracted from whole blood, followed by *BTK* gene amplification by PCR. The PCR product is purified from unincorporated primers and nucleotides by enzymatic digestion and sequenced in both directions using sequencing primers and fluorescent dye-terminator chemistry. Sequencing products are separated on an automated sequencer and trace files are analyzed for variations in the exons and intron/exon boundaries of all 19 exons using specialized variant detection software and visual inspection.(Unpublished Mayo method)

The Bruton tyrosine kinase (Btk) protein expression flow cytometry assay is carried out with a whole blood sample. The cells in the blood are stained with antihuman CD20 (B cells) and CD14 (monocytes) antibodies, followed by RBC lysis (using a premade Lysis buffer), cell fixation and permeabilization to prepare the cell membrane for the antihuman Btk antibody. After the permeabilization step, the cells are stained for intracellular Btk using an antihuman Btk-fluorescent preconjugated antibody from BD Biosciences. After the staining and wash process, the cells are resuspended in 500 mcL of BD FACS stain buffer in the final step of the assay and the sample is analyzed by multiparametric flow cytometry.(Unpublished Mayo method; 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)

PDF Report

No

Specimen Retention Time

Whole Blood: 4 days Extracted DNA: 2 months

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

CPT Code Information

81406-Bruton Tyrosine Kinase (*BTK*) Genotype, Full Gene Sequence

88184-Bruton Tyrosine Kinase (Btk), Protein Expression, Flow Cytometry, Blood

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
BTKFP	BTK Full-Gene Panel, B	94241-7

Result ID	Reporting Name	LOINC®
89011	Btk Protein Flow, B	75708-8
BTKSQ	BTK, Full Gene Sequencing	Bill only; no result
29305	BTK Full Gene Result	82939-0
45486	BTK Full Gene Interpretation	69047-9
45487	Reviewed By	18771-6