Test Definition: BTKS

BTK, Full Gene Sequence

Overview

Useful For

Confirming a diagnosis of X-linked agammaglobulinemia (XLA) in male 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 female relatives (of male XLA patients) who do not demonstrate carrier phenotype by Btk flow cytometry

Profile Information

Test ID	Reporting Name	Available Separately	Always Performed
BTKSP	BTK, Full Gene Sequence	No	Yes
BTKSQ	BTK, Full Gene Sequencing	No	Yes

Special Instructions

- Informed Consent for Genetic Testing
- Bruton Tyrosine Kinase (BTK) Genotype Patient Information
- Multiple Whole Blood EDTA Genotype Tests
- Informed Consent for Genetic Testing (Spanish)

Method Name

Polymerase Chain Reaction (PCR) Followed by DNA Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Advisory Information

The preferred test for confirming a diagnosis of X-linked agammaglobulinemia in males and identifying carrier females is BTKFP / Bruton Tyrosine Kinase (*BTK*) Genotype and Protein Analysis, Full Gene Sequence and Flow Cytometry

For cases where the differential diagnosis remains broad, *BTK* may be evaluated as part of a larger genetic panel, see BCLGP / B-Cell Deficiency Primary Immunodeficiency (PID) Gene Panel.

Necessary Information

1. Bruton Tyrosine Kinase (*BTK*) Genotype Patient Information **(T620) is required,** see Special Instructions. Testing may proceed without the patient information however it aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to complete the form and send it with the specimen.

2. Include physician name and phone number with specimen.

Specimen Required

Multiple whole blood EDTA genotype tests can be performed on a single specimen after a single extraction. See Multiple Whole Blood EDTA Genotype Tests in Special Instructions for a list of tests that can be ordered together.

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions: Send specimen in original tube.

Forms

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 in Special Instructions:

-Informed Consent for Genetic Testing (T576)

-Informed Consent for Genetic Testing-Spanish (T826)

Specimen Minimum Volume

0.35 mL

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

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

Clinical and 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 diagnostic clinical 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.

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 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. Over 800 unique variants in *BTK* have been detected by full gene sequencing and are listed in BTKbase, a database for *BTK* variants (http://structure.bmc.lu.se/idbase/BTKbase/).(5)Â Missense variants account for approximately 33% of unique variants, nonsense variants 13%, frameshift 25%, in-frame deletions and insertions 4%, large deletions 3% to 5%, and intronic and complex variants make up the remainder. Patients with a large deletion spanning the *BTK* gene may also impact the adjacent *TIMM8A* gene (also known as *DDP*) resulting in both XLA and deafness-dystonia-optic neuropathy syndrome (DDS or Mohr-Tranebjaerg syndrome). Genotype-phenotype correlations have not been completely defined for *BTK*, but it is clear that nonsense and frameshift variants are overrepresented 4-fold compared with substitutions, which indicates that the latter may be tolerated without causing a phenotype or with a milder phenotype or later age at presentation. Some individuals present within the first 2 years 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 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). 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.

The preferred approach for confirming a diagnosis of XLA in males and identifying carrier females 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 [Btk], 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 varaint 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

A patient-specific interpretive report is provided.

Cautions

Rare polymorphisms 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.

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

This method will not detect variants that occur in intronic (other than exon-intron boundaries) and regulatory regions of the Bruton tyrosine kinase gene (*BTK*) gene or large rearrangement-type variants.Â This assay is not designed to detect large deletions.

If the full gene sequencing does not match the clinical impression, flow cytometry should be performed to assess expression of Btk protein (BTK / Bruton Tyrosine Kinase [Btk], Protein Expression, Flow Cytometry, Blood). Large deletions or rearrangements will affect protein expression, and the absence of Btk protein on monocytes can be determined by flow cytometry.

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 Jan 29;72(2):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 healthy children. Pediatr Res 2002 Feb;51(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 Feb;203:216-234

4. Lindvall JM, Blomberg KE, Valiaho J, et al: BrutonÃ¢â‚¬â„¢s tyrosine kinase: cell biology, sequence conservation, mutation spectrum, siRNA modifications, and expression profiling. Immunol Rev 2005 Feb;203:200-215

5. Valiaho J, Smith CI, Vihinen M: BTKbase: the mutation database for X-linked agammaglobulinemia. Hum Mutat 2006 Dec;27(12):1209-1217

6. 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 2004 Jan 1;103(1):185-187

Performance

Method Description

Genomic DNA is first extracted from whole blood, followed by Bruton tyrosine kinase gene (*BTK*) 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 mutation detection softwareÂ and visual inspection.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday; 8 a.m.

Analytic Time

28 days

Maximum Laboratory Time

42 days

Specimen Retention Time

Whole Blood: 2 weeks Extracted DNA: 2 months

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

81406

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
BTKS	BTK, Full Gene Sequence	94241-7

Result ID	Test Result Name	Result LOINC Value
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