Overview

Useful For

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

Because genotype-phenotype correlations are important, the preferred test for confirming a diagnosis of XLA in males and identifying carrier females is:

-BTKFP / Bruton Tyrosine Kinase (BTK) Genotype and Protein Analysis, Full Gene Sequence and Flow Cytometry

-In families where a BTK mutation has already been identified, order BTKMP / Bruton Tyrosine Kinase (BTK) Genotype and Protein Analysis, Known Mutation Sequencing and Flow Cytometry

Method Name

FlowCytometry

NY State Available

No

Specimen

Specimen Type

Whole Blood EDTA

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 and phone number are required.

Specimen Required

For serial monitoring, we recommend that specimen draws be performed at the same time of day.

Container/Tube: Lavender top (EDTA)

Specimen Volume: 4 mL

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

Specimen Minimum Volume

2 mL

Reject Due To
Clinical and 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, adult male patients 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 mutations and confirming a diagnosis of XLA, it is labor intensive and expensive. 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 XLA patients 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 mutations 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 mutations 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 XLA patients 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 mutations within the gene. Females are typically carriers and asymptomatic. Testing in adult females 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 mutations.

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 mutation within the **BTK** gene have
normal protein expression (again related to the position of the mutation 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, which includes both flow cytometry and gene sequencing to confirm the presence of a BTK mutation. If a familial mutation has already been identified, then BTKMP / Bruton Tyrosine Kinase (BTK) Genotype and Protein Analysis, Known Mutation Sequencing and Flow Cytometry should be ordered.

Reference Values

Present

Bruton tyrosine kinase (Btk) 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. 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 or BTKK / Bruton Tyrosine Kinase (BTK) Genotype, Known Mutation) 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 (XLA), 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 mutant X-chromosome inactivation) or there is dominant expression of the normal protein in the presence of 1 copy of a mutation.

Cautions

This test is typically not indicated in adult males (>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) or females beyond child-bearing age. For questions about appropriate test selection, call 800-533-1710.

The flow cytometry screening assay is likely to detect the majority of X-linked agammaglobulinemia (XLA) patients with 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 which can only be detected by BTK gene sequencing. The ability to identify carrier females 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 carrier females.

It is also important to note that there are XLA patients with mothers who have normal Btk protein expression by flow cytometry and normal BTK genotyping, and the mutation in the patient is a result of de novo mutations 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 mutant X chromosome.

Clinical Reference


Performance

Method Description

The Bruton tyrosine kinase (Btk) protein expression screening 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, which is followed by red blood cell 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 antihuman Btk-fluorescent preconjugated antibody (BD Biosciences). After the staining and wash process, the cells are fixed and 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

Day(s) and Time(s) Test Performed

Monday through Friday

Do not send specimen after Thursday. Specimen must be received by 10 a.m. on Friday.

Analytic Time

3 days

Maximum Laboratory Time

4 days

Specimen Retention Time

4 days

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.
Test Definition: BTK
Btk Protein Flow, B

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 U.S. Food and Drug Administration.

CPT Code Information
88184

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTK</td>
<td>Btk Protein Flow, B</td>
<td>75708-8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>89011</td>
<td>Btk Protein Flow, B</td>
<td>75708-8</td>
</tr>
</tbody>
</table>