BTK Protein Expression by Flow Cytometry

Indications for Ordering
Initial screening for suspected primary agammaglobulinemia

Test Description
Whole blood specimen is stained with CD14 and CD20 to identify monocytes and B cells, respectively
• Followed by intracellular staining with fluorescently labeled anti-Bruton tyrosine kinase (BTK) and isotype control antibodies
  o Flow cytometry is used to detect BTK protein expression in monocytes and B cells

Tests to Consider
Primary test
Bruton Tyrosine Kinase (BTK) Protein Expression by Flow Cytometry 2012002
• Preferred test for initial screening for individuals with clinical suspicion of X-linked agammaglobulinemia/hypogammaglobulinemia

Related tests
Initial screening for immunodeficiency
• CBC with Platelet Count and Automated Differential 0040003
• Immunoglobulins (IgA, IgG, IgM), Quantitative 0050630
• Lymphocyte Subset Panel 7 – Congenital Immunodeficiencies 0095899
• Lymphocyte Antigen and Mitogen Proliferation Panel with Cytokine Response 2013117
• Familial Mutation, Targeted Sequencing 2001961
  o Useful when a pathogenic familial variant identifiable by sequencing is known

Confirm suspected agammaglobulinemia
Primary Antibody Deficiency Panel, Sequencing and Deletion/Duplication 2011156
• Preferred genetic test for individual with clinical phenotype of primary antibody deficiency (eg, common variable immunodeficiency)

Disease Overview
Prevalence
• X-linked agammaglobulinemia (XLA) – ~1/250,000-700,000

Age of onset – usually within the first two years of life

Symptoms
• Recurrent or chronic infections
  o Encapsulated pyogenic bacteria
  o Enteroviruses
• Neutropenia
• Pneumonia/empyema
• Gastroenteritis
• Otitis media
• Meningitis
• Sepsis

Diagnostic issues
• BTK gene mutations are responsible for XLA
• Deficiency or partial deficiency of BTK protein expression is surrogate marker for lack of functioning BTK gene
• Screening for BTK protein expression is recommended prior to genetic testing for phenotype/genotype correlations

Pathophysiology
• Most mutations of BTK gene result in absence of BTK protein, which is essential for maturation of B cells
• Absence or lack of functional BTK protein leads to a lack of immunoglobulins
  o Causes increased susceptibility to infections
  o Occurs almost exclusively in males

Genetics
Gene – BTK
Inheritance – X-linked
Mutations – >600 known mutations in BTK gene cause XLA

Test Interpretation
Results
• Normal – suggests the presence of BTK protein
• Absent – consistent with XLA in males
• Reduced – suggests XLA in males
• Mosaic – two populations (one with normal BTK expression and one with abnormal BTK expression)
  o Suggests carrier status for XLA in females
Limitations

Normal expression of BTK protein
- Occurs in 20-30% of patients with XLA due to truncated or inactive BTK protein with abnormal function
  - Genetic analysis is recommended
- Does not exclude mutations or defective protein function
- Does not rule out XLA in males
- Does not rule out XLA carrier status in females