Chronic Granulomatous Disease

**Indications for Ordering**

- Confirm a clinical or laboratory diagnosis of chronic granulomatous disease (CGD)
- Assess carrier status for CGD
- Predictive testing for unaffected at-risk relatives

**Test Descriptions**

**Neutrophil Oxidative Burst Assay (DHR)**
- Semiquantitative flow cytometry

**CYBB Sequencing**
- Bidirectional sequencing of the CYBB coding region and intron-exon boundaries

**CYBB Sequencing and NCF1 Exon 2 GT Deletion**
- Bidirectional sequencing of the CYBB coding region and intron-exon boundaries
- Polymerase Chain Reaction/High-Resolution Melt Analysis to assess for the common pathogenic NCF1 c.75_76delGT variant

**Tests to Consider**

**Typical testing strategy**
- A provisional diagnosis of CGD is made using functional tests to detect the absence or reduction of oxidase activity in activated neutrophils (eg, respiratory burst test)
- If abnormal oxidase function is noted, molecular testing to confirm the causative variant(s) is necessary for
  - Diagnostic confirmation
  - Genetic counseling
  - Prenatal diagnosis

**Primary tests**

**Neutrophil Oxidative Burst Assay (DHR)** 0096657
- Aid in screening for CGD

**Chronic Granulomatous Disease Panel (CYBB Sequencing and NCF1 Exon 2 GT Deletion)** 3000544
- Preferred test to assess common molecular causes of CGD

**Related tests**

**Chronic Granulomatous Disease, X-Linked (CYBB) Sequencing** 3000541
- Molecular test to confirm a diagnosis or assess carrier status for X-linked CGD

**Disease Overview**

**Incidence** – 1/250,000 births in the U.S.
- X-linked CGD
  - CYBB – 60-70% of all cases
- Autosomal recessive CGD
  - NCF1 – 25% of cases
  - CYBA – <5% of cases
  - NCF2 – <5% of cases
  - NCF4 – very rare

**Pathophysiology**
- Primary immunodeficiency disorder that results from changes within genes encoding the essential subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, leading to
  - Recurrent, severe bacterial and fungal infections
  - Dysregulated inflammatory responses leading to granulomas at infection sites

**Common infectious agents include**
- *Staphylococcus aureus*
- *Burkholderia cepacia*
- *Serratia marcescens*
- *Nocardia* spp
- *Aspergillus* spp

**Chronic Granulomatous Disease (NCF1) Exon 2 GT Deletion** 2006366
- Tests for a common pathogenic NCF1 variant associated with autosomal recessive CGD

**Familial Mutation, Targeted Sequencing** 2001961
- Useful when a pathogenic familial variant identifiable by sequencing is known
Symptoms
- Recurrent severe bacterial and fungal infections of various organs
  - Lymph nodes
  - Liver
  - Lungs
  - Bones
  - Visceral organs
- Granulomas form at infection sites
- Other findings
  - Poor wound healing
  - Hypergammaglobulinemia
  - Splenomegaly
  - Chorioretinitis
  - Colitis/enteritis
  - Obstructions of urinary tract or gastric outlet
- Males with classic X-linked CGD are typically diagnosed before 3 years of age
  - Less severe phenotypes have been observed and may be diagnosed later in life
- Approximately 50% of carrier females of X-linked disease may have mild symptoms, including
  - Photosensitivity
  - Recurrent mouth ulcers
- Females with skewed X-chromosome inactivation may have severe disease presentation
- Individuals with X-linked CGD typically have earlier onset and more severe disease than individuals with variant X-linked or autosomal recessive CGD

Diagnostic issues
- Early diagnosis is essential – disease management relies on lifelong antibiotic and antifungal prophylaxis
- Disease severity can be estimated by the level of NADPH oxidase activity associated with a particular CYBB gene variant
  - Genetic or environmental modifiers may result in variable clinical outcomes

Genetics

Genes associated with autosomal recessive CGD
- NCF1 – encodes p47-phox
  - GT deletion in exon 2 (c.75_76delGT) accounts for ~85% of causative variants in NCF1 (Vázquez 2001)
- CYBA – encodes p22-phox
- NCF2 – encodes p67-phox
- NCF4 – encodes p40-phox

Test Interpretation

Chronic Granulomatous Disease Panel (CYBB Sequencing and NCF1 Exon 2 GT Deletion)

Sensitivity/specificity
- Clinical sensitivity – Up to 78% for CGD (Roos, 2010a; Roos, 2010b; Vázquez, 2001)
- Analytical sensitivity – 99% for CYBB or homozygous NCF1 GT deletion; 90% for heterozygous NCF1 GT deletion
- Analytical specificity – 99%

Results
- Positive – pathogenic variant was detected
  - CYBB variant
    - In symptomatic male – confirms X-linked CGD
    - In asymptomatic female – confirms carrier status for X-linked CGD
  - NCF1 GT deletion (c.75_76delGT)
    - 2 copies confirms autosomal recessive CGD
    - 1 copy confirms carrier status for autosomal recessive CGD
- Negative – CYBB gene pathogenic variant and the common pathogenic NCF1 GT deletion not detected
  - Reduces, but does not eliminate, the possibility of CGD
- Inconclusive – gene sequencing may detect novel variants of unknown clinical significance

Limitations
- Diagnostic errors can occur due to rare sequence variations
- Regulatory region variants, deep intronic variants, and large duplications in CYBB will not be detected in patients of either sex
- Large CYBB gene deletions will not be detected in females
- Variants in NCF1 other than c.75_76delGT are not evaluated
- Lack of detection of NCF1 c.75_76delGT does not rule out carrier status
  - Due to potential recombination between NCF1 and its pseudogenes, false negative results may occur for c.75_75delGT heterozygotes
- This assay does not interrogate all genes associated with CGD (eg, CYBA, NCF,2 and NCF4)
References