

Severe Combined Immunodeficiency Panel by next-generation sequencing (NGS)

Genes Tested

<i>ADA</i>	<i>CD3D</i>	<i>CD3E</i>	<i>DCLRE1C</i> (<i>Artemis</i>)	<i>FOXP1</i>
<i>IL2RG</i>	<i>IL7R</i>	<i>JAK3</i>	<i>LIG4</i>	<i>NHEJ1</i>
<i>ORAI1</i>	<i>PNP</i>	<i>PTPRC</i>	<i>RAG1</i>	<i>RAG2</i>
<i>RMRP</i>	<i>STAT5B</i>	<i>STIM1</i>	<i>TBX1</i>	<i>ZAP70</i>

Disorder: This panel detects the most common causes of severe combined immunodeficiency (SCID) and Omenn syndrome, as well as cartilage hair hypoplasia-anauxetic dysplasia spectrum and velocardiofacial syndrome secondary to mutations in *RMRP* and *TBX1* respectively.

Severe Combined Immunodeficiency is a genetically heterogeneous disorder of T lymphocyte development and adaptive immunity. The estimated prevalence of SCID is 1 in 50,000 births with a higher prevalence in males. Symptoms usually begin between three and six months of age and include severe infections, chronic diarrhea, and failure to thrive. Laboratory screening tests consistent with the diagnosis of SCID include abnormal mitogen stimulation, abnormal lymphocyte subsets and abnormal TREC assay. SCID is considered a pediatric emergency and if untreated, is often fatal by six to twelve months of age. However, many patients have a favorable prognosis with early diagnosis and bone marrow stem cell transplantation.

The most common type of SCID is caused by X-linked mutations in the *IL2RG* gene and accounts for 80% of SCID in male patients. The remaining known causes of SCID are caused by autosomal recessive mutations. All genes associated with SCID cause T cell deficiency, but B cell and NK cell deficiency varies depending on the causative gene. This SCID panel is expected to identify > 85% of the genetic causes of SCID.

Omenn syndrome is caused by hypomorphic mutations in several genes associated with SCID which allow for low levels of T lymphocyte development. Along with immunodeficiency, patients with Omenn syndrome typically have severe erythroderma, desquamation, alopecia, lymphadenopathy, eosinophilia and elevated levels of IgE. Omenn syndrome has been reported in patients with *RAG1*, *RAG2*, *DCLRE1C*, *IL7RA*, *RMRP*, *IL2RG*, *LIG4* and *ADA* mutations.

The **cartilage-hair hypoplasia-anauxetic dysplasia** spectrum due to mutations in the *RMRP* gene is characterized by short stature with mild metaphyseal dysplasia in its mildest form. Cartilage-hair hypoplasia is an intermediate phenotype characterized by short stature with moderate metaphyseal dysplasia and hypotrichosis and may also be associated with Hirschsprung disease, B cell and/or T cell immunodeficiency and hematologic abnormalities and malignancies. Anauxetic dysplasia represents the most severe end of the spectrum of mutations in *RMRP*. Genotype/phenotype correlations have been proposed. In general, the degree of rRNA cleavage decrease strongly correlates with the degree of bone dysplasia [see Thiel & Rauch 2011].



Human Genetics

Cytogenetics and Molecular Genetics Laboratories
CLIA#: 36D0656333
Phone: (513) 636-4474
Fax: (513) 636-4373
www.cchmc.org/genetics



Disorder continued:

Velocardiofacial syndrome (VCF) is a well characterized genetic syndrome associated with cleft palate, cardiac defects, typical facial features, immunodeficiency secondary to thymic hypoplasia, and intellectual, behavioral and/or psychiatric disabilities. 95% of individuals with VCF have a small deletion of chromosome 22q11, which includes the *TBX1* gene, while <5% have mutations within the *TBX1* gene itself. Therefore, FISH testing for the 22q11 microdeletion is usually indicated prior to molecular testing for *TBX1* mutations.

Indications: Confirmation of diagnosis in a patient with suspected:

- SCID
- Omenn syndrome
- Microcephaly and immune deficiency
- Velocardiofacial syndrome in the absence of a 22q deletion by FISH analysis
- Cartilage hair hypoplasia-anauxetic dysplasia spectrum
- T cell deficiency of unknown etiology.

Specimen: At least 5 mLs whole blood in a lavender top (EDTA) tube. Label tube with patient's name, birth date, and date of collection.

Testing Methodology: This test is performed by enrichment of the exons, flanking intronic and un-translated regions (5' and 3') of the genes specified above using microdroplet PCR technology followed by next-generation sequencing with > 40 fold coverage at every target base. All pathogenic and novel variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing.

Sensitivity:

Clinical Sensitivity: Sequencing detects 60-99% of the reported mutations in these genes using this testing methodology. Large exonic deletions have been reported in *ADA*, *DCLRE1C*, *IL2RG*, *JAK3*, *NHEJ1*, *PTPRC*, *RAG1*, *RAG2*, *RMRP*, *STAT5B* and *TBX1*. Deletion/duplication analysis may be indicated as a follow-up test in patients with a single mutation in one of these genes, or in patients with normal SCID panel analysis.

Analytical Sensitivity: The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Mutations in regulatory regions or other untranslated regions are not detected by this test. Large deletions involving entire single exons or multiple exons, large insertions and other complex genetic events have been reported in many of these genes and will not be identified using this test methodology. Rare primer site variants may lead to erroneous results.

Note: Single gene sequencing is available for all genes in the panel.

Turn-Around Time:

- 56 days for NGS of the panel
- 90 days for analysis of any gene on the panel by Sanger sequencing, except for the following: *IL2RG* (30 days)

CPT Codes:

- Severe Combined Immunodeficiency Panel by NGS 81405, 81479x19
- Single gene sequencing of any gene in this panel 81479

Cost: Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.



Results: Results will be reported to the referring physician or health care provider as specified on the test requisition.

Shipping Instructions

Please enclose **test requisition** with sample. **All information must be completed before sample can be processed.** Place samples in Styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

- Aloj, G, et al. (2012). Int Rev Immunol, 31(1), 43-65.*
- Buck, D, et al. (2006). Cell, 124(2), 287-299.*
- Buckley, RH. (2004). J Clin Invest, 114(10), 1409-1411.*
- Chinnadurai, S., et al. (2012). Curr Opin Otolaryngol Head Neck Surg, 20(6), 502-50.*
- El Omari, K., et al. (2011). Proteins.*
- Feske, S, et al. (2010). Clin Immunol, 135(2), 169-182.*
- Fischer, A, et al. (2005). Immunol Rev, 203, 98-109.*
- Fischer, A, et al. (2005). Curr Opin Allergy Clin Immunol, 5(6), 491-495.*
- Gaspar, HB, et al. (2009). Blood, 114(17), 3524-3532.*
- Guo, T, et al. (2011). Hum Mutat, 32(11), 1278-1289.*
- Herman, S. B., et al. (2012). Am J Med Genet A, 158A(11), 2781-2787. Mazzucchelli, RI, et al. (2012). Semin Immunol, 24(3), 225-230.*
- Adam (Eds.), GeneReviews. Seattle (WA).*
- McDonald-McGinn, D. M., et al. (1993). 22q11.2 Deletion Syndrome. In R. A. Pagon, T. D. Bird, C. R. Dolan, K. Stephens & M. P. Adam (Eds.), GeneReviews. Seattle (WA).*
- Moshous, D, et al. (2001). Cell, 105(2), 177-186.*
- Nadeau, K, et al. (2011). J Pediatr, 158(5), 701-708.*
- Niehues, T, et al. (2010). Clin Immunol, 135(2), 183-192.*
- Notarangelo, LD, et al. (2001). Hum Mutat, 18(4), 255-263.*
- Pignata, C, et al. (2009). Adv Exp Med Biol, 665, 195-206.*
- Roifman, C. M., et al. (2012). J Allergy Clin Immunol, 130(1), 177-183.*
- Sponzilli, I, et al. (2011). Acta Biomed, 82(1), 5-13.*
- Tewhey, R., et al. (2009). Nat Biotechnol, 27(11); 1025-1031.*
- Thiel, C & Rauch, A. (2011) Best Practice & Research Clinical Endocrinology & Metabolism 25:131-142.*