

Fanconi Anemia Panel by next-generation sequencing (NGS)

Genes Tested

<i>FANCA</i>	<i>FANCB</i>	<i>FANCC</i>	<i>BRCA2</i> (<i>FANCD1</i>)
<i>FANCD2</i>	<i>FANCE</i>	<i>FANCF</i>	<i>FANCG</i>
<i>FANCI</i>	<i>FANCI</i> (<i>BRIP1</i>)	<i>FANCL</i>	<i>FANCM</i>
<i>PALB2</i> (<i>FANCN</i>)	<i>SLX4</i> (<i>FANCP</i>)	<i>RAD51C</i> (<i>FANCO</i>)	<i>ERCC4</i> (<i>FANCQ</i>)

Disorder: Fanconi Anemia (FA) is a rare, inherited chromosome - instability syndrome, estimated to occur in 1:100,000 live births. However, its prevalence is much higher in some populations including Ashkenazi Jewish, Spanish Gypsy, and black South African.

A unique characteristic that distinguishes FA from other chromosome breakage syndromes is the cellular hypersensitivity to DNA cross-linking agents causing chromosome breakage. Much of the function of the FA proteins in normal cells is unclear. Spontaneous reversion of a pathogenic allele to wild type (or correction of cellular defect with a second mutation) in patients who are homozygous for FA mutations has been reported.

Patients with FA have varied clinical manifestations, most commonly progressive bone marrow at a median age of five (5) years, progressive pancytopenia and congenital malformations including short stature, radial aplasia, urinary tract abnormalities, hyperpigmentation, and/or developmental delay. FA patients also have a predisposition to cancer, particularly acute myeloid leukemia, as well as an increased risk of developing solid tumors in the head, neck, skin, GI tract, and genital tract. The symptoms of FA are highly variable, even among individuals within the same family or among patients within the same complementation group. Treatment depends on the symptoms experienced by the individual patient.

Mutations which result in premature termination of the *FANCB* protein are typically associated with a severe VACTERL-H phenotype which includes ventriculomegaly/hydrocephalus, radial ray defects with aplastic or hypoplastic thumbs, urinary tract abnormalities, vertebral defects, hypogonadism, gastrointestinal atresia and pre- or postnatal growth retardation, in addition to abnormal chromosome breakage and development of anemia.

Genetics: FA is usually inherited as an autosomal recessive condition, except for Fanconi anemia type B that is X-linked. Biallelic mutations are identified in approximately 85% of patients with FA. Fanconi anemia is genetically heterogeneous and mutations in 16 different genes have been identified in FA patients to date. Mutations in three genes (*FANCA*, *FANCC*, and *FANCG*) account for disease in the majority of patients. Heterozygous carriers of inactivating mutations in *BRCA2*, *PALB2* and *RAD51C* at *SLX4* are at some increased risk of developing breast and/or ovarian cancers.



Human Genetics

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Indications:

FA Panel by NGS:

- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of Fanconi anemia
- Carrier identification in individuals with a family history of FA of unknown genetic basis.

Gene Specific Sequencing:

- Confirmation of genetic diagnosis in a patient with FA and in whom complementation studies suggest a specific genetic diagnosis.

Mutation Specific Analysis:

- Presymptomatic testing of at-risk siblings for medical management and prior to bone marrow donation
- Carrier identification in individuals in whom specific mutation(s) have been identified in the proband with FA
- Prenatal diagnosis of an at-risk fetus, after confirmation of biallelic mutations in the parents (by prior arrangement only).

Ancillary Testing: Chromosome breakage studies should be performed in most patients prior to mutation analysis. Complementation group assay is a useful adjunct to mutation analysis in patients with (suspected) Fanconi anemia and may either be initiated prior to genetic testing in order to identify a specific complementation group for targeted molecular analysis, or as follow-up testing in patients with abnormal or ambiguous molecular genetics results. For more information about these tests, please call 513-636-4474.

Specimen: FA Panel by NGS: At least 5 mLs whole blood in a lavender top (EDTA) tube.

Gene Specific Sequencing or Mutation Specific

Analysis: At least 3 mLs whole blood in a lavender top (EDTA) tube.

Note: Saliva samples are required for analysis in patients who have undergone bone marrow transplantation and may facilitate DNA isolation in patients undergoing chemotherapy or in individuals with leukopenia. Please call 513-636-4474 for a free saliva collection kit.

Testing Methodology:

FA Panel by NGS: This test is performed by enrichment of the exons, flanking intronic and un-translated regions (5' and 3') of the specified genes specified 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 significance, as determined bioinformatically, are confirmed by Sanger sequencing. Individual Sanger-based sequencing, either mutation specific or PCR-based sequencing of the entire coding region and intron/exon boundaries, of each gene on this panel is also available.

Gene Specific Sequencing/ Mutation Specific Analysis: Sanger sequencing following PCR amplification of the specified coding and exon/intron boundaries of the specified gene. Single gene sequencing is available for every gene in the panel.

Sensitivity:

Clinical Sensitivity: DNA sequencing detects 80-99% of the reported mutations in these genes, depending on genetic subtype. Large exonic deletions are common in *FANCA* and have been reported in most of the other genes on this panel. Deletion/duplication analysis may be indicated as a follow-up test in symptomatic patients with a normal sequencing result or a single (heterozygous) mutation in one of the genes on the panel.

Analytical Sensitivity: The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions (<10 bp) and insertions in the regions analyzed. Mutations in regulatory regions or other untranslated regions are not detected by this test. Deletions and insertions of >10 bp, as well as other complex genetic events have been reported in many of the genes on this panel 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:

- Up to 12 weeks for NGS of the entire Fanconi Anemia Panel
- 4-12 weeks for analysis of any gene by Sanger sequencing

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

CPT Codes:

- Fanconi Anemia Panel by NGS 81406, 81479x16
- Single gene sequencing of *PALB2* 81406
- Single gene sequencing of any other gene in this panel 81479
- *FANCC* IVS4+4 A>T common Ashkenazi mutation 81242
- Family Study 81403

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 1013
Cincinnati, OH 45229
513-636-4474

References:

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- Tewhey, R., J. B. Warner, et al. (2009). "Microdroplet-based PCR enrichment for large-scale targeted sequencing." *Nat Biotechnol* 27(11): 1025-1031.
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