Cincinnati Children's

Cytogenetic Laboratory
Molecular Genetics Laboratory
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Fanconi Anemia

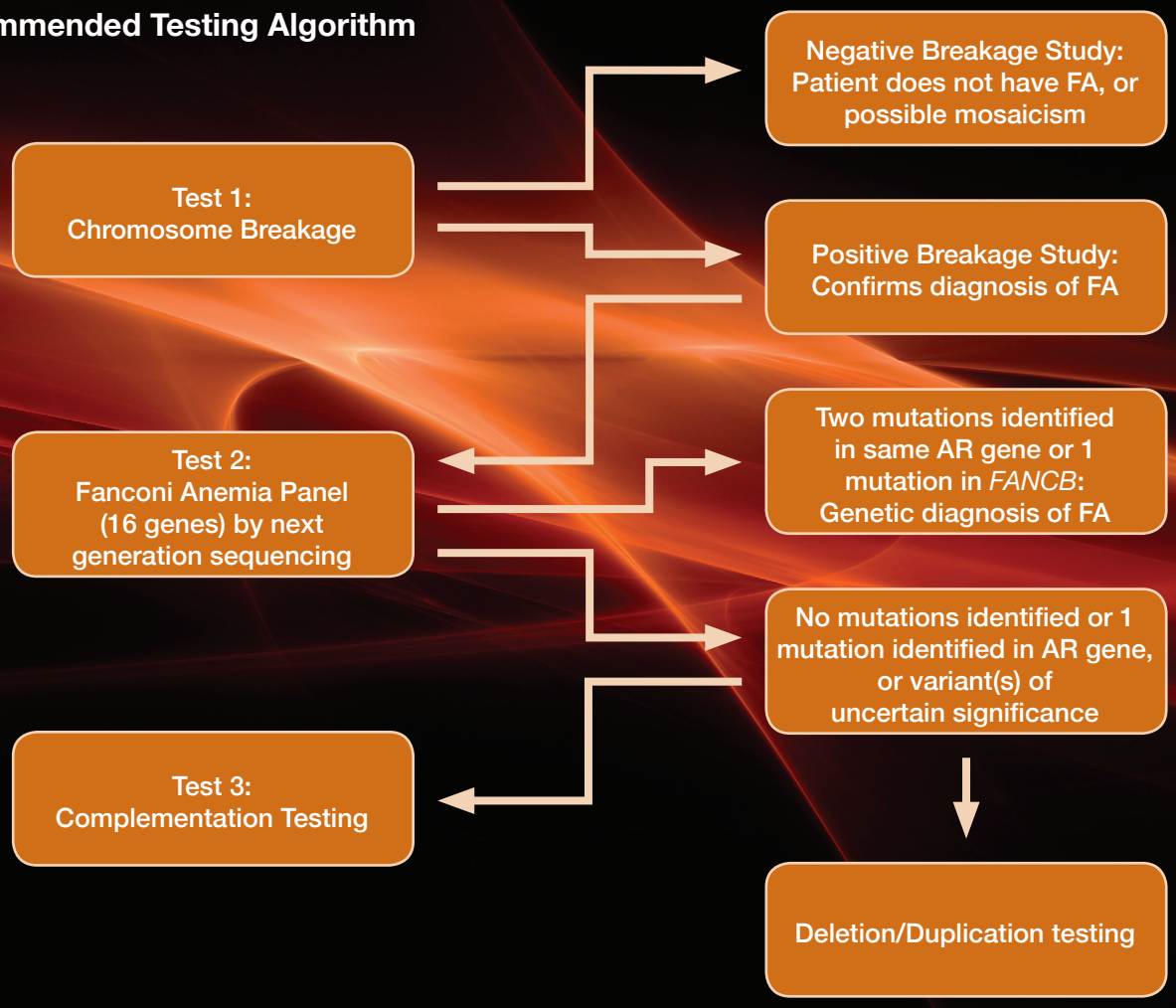
Fanconi anemia (FA) is a rare, inherited chromosome instability syndrome, estimated to occur in 1 in 100,000 live births. Patients with FA have varied clinical manifestations. Most patients experience bone marrow failure at a median age of five years. Progressive pancytopenia and congenital malformations, including short stature, radial aplasia, urinary tract abnormalities, hyperpigmentation, and developmental delay are common symptoms. FA is associated with a predisposition to cancer, particularly acute myeloid leukemia and an increased risk of developing solid tumors. The symptoms of FA are highly variable; treatment depends on the symptoms experienced by the individual patient.

Indications:
Testing for Fanconi anemia is indicated in young patients with aplastic anemia, arm and/or thumb, cardiac, central nervous system, genitourinary, kidney, and/or skeletal system anomalies, hyper-pigmentation, small size, and/or bleeding disorders.

For more information call 513-636-4474 or visit www.cincinnatichildrens.org/FanconiTesting

We also offer testing for other chromosome breakage disorders including Sister Chromatid Exchange (SCE) analysis for Bloom syndrome. Please see our website for additional information.

Recommended Testing Algorithm



This is the suggested testing algorithm. Please note that any test can be requested in any order.

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Tests 1, 2, and 3 can be ordered individually, concurrently, or sequentially
Please call 1-866-450-4198 with any billing or CPT questions.

Test 1: Chromosome Breakage – When exposed to DNA cross-linkage agents the chromosomes of FA patients will break and form abnormal patterns.

Methodology: Lymphocytes are stimulated and cultured from peripheral blood. Baseline breakage, without a DNA damaging agent, is recorded. Then DNA damaging agents Mitomycin C (MMC) and Diepoxybutane(DEB) are added and breakage is recorded. Twenty-five baseline metaphase cells and 50 cells each from MMC and DEB culture conditions are evaluated concurrently against a control.

Sensitivity/Specificity: Chromosome breakage in the presence of DEB is the most specific tool when testing for FA and is considered diagnostic.

Turn around: 14 days (additional time may be needed for fibroblast culture)

Specimen: 5-10mL peripheral blood in sodium-heparin (green-top) OR 5-10mL bone marrow in sodium-heparin (green-top) OR skin biopsy OR (2) T25 flasks of primary fibroblast cell line.

Test 2: Genetic Testing – For patients with a positive chromosome breakage study, the Fanconi Anemia Panel is used to identify the causative mutation(s). This 16 gene panel uses next generation sequencing (NGS) to analyze *BRCA2, BRIP1, ERCC4, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, PALB2, RAD51C* and *SLX4.* Single gene sequencing of each gene on the panel is also available, as is mutation specific analysis for the *FANCC* common IVS4+4 A>T mutation in patients of Ashkenazi Jewish descent.

Methodology: The Fanconi Anemia Panel is performed by enrichment of the exons, flanking intronic regions, and un-translated regions of the genes specified using microdroplet PCR followed by NGS with > 20 fold coverage at every target base. All pathogenic and novel variants, as well as variants of unknown significance, are confirmed by Sanger sequencing. Individual gene sequencing is performed by PCR-based Sanger sequencing of the entire coding regions and intron/exon boundaries of the specified gene. The *FANCC* common Ashkenazi mutation is detected by mutation-specific PCR.

Sensitivity/Specificity: Greater than 85% of patients with a clinical diagnosis of Fanconi anemia will have mutations in the genes included in the Fanconi Anemia Panel. Large deletions have been reported in most of the FA genes and are not detected by this panel. Targeted deletion and duplication analysis of each gene on this panel is clinically available at an additional charge.

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

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. Please call 513-636-4474 for other specimen types.

Test 3: Complementation – For patients with FA confirmed by positive DEB/MMC induced chromosome breakage studies, complementation analysis seeks to identify the patient's complementation group.

Methodology: Tier 1: Establish Cell line. Tier 2: Complementation analysis begins with testing for the most common groups A, C and G. Tier 3: If indicated, testing continues with complementation analysis for groups E, F and L (and B for male patients). Complementation analysis for *FANCI* is available for skin fibroblast samples only. A complementation group is identified when drug induced cell cycle arrest is corrected by addition of a specific FA gene vector.

Sensitivity/Specificity: Groups A, C, G, E, F, L and B account for 90% of FA patients in the US. Approximately 15-20% of cell lines derived from the blood of patients with FA are not sensitive to Melphalan and will not yield complementation results (skin biopsy may be required). In this lab, a complementation group has been identified in >60% of viable, sensitive cell lines.

Turn around: If tiers 2 through 3 are done sequentially, TAT is 3-6 months for groups A, C, and G (85%) or approximately a year for groups E, F, L and B. If tiers are done concurrently, total TAT is 6-9 months.

Specimen: 3ml of peripheral blood collected in ACD (yellow top tube. Fresh sample may be needed for reflex.) OR skin biopsy OR EBV transformed lymphoblastic cell line OR primary fibroblast cell line.