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**

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*Fanconi Anemia*

1. **Test 1:** Chromosome Breakage
   - Negative Breakage Study: Patient does not have FA, or possible mosaicism
   - Positive Breakage Study: Confirms diagnosis of FA

2. **Test 2:** Fanconi Anemia Panel (16 genes) by Next Generation Sequencing
   - Two mutations identified in same AR gene or 1 mutation in FANCB: Genetic diagnosis of FA
   - No mutations identified or 1 mutation identified in AR gene, or variant(s) of uncertain significance

*Complementation Testing (available for research/investigational purposes only)

- Deletion/Duplication testing

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

*Contact 513-636-5998 for details regarding complementation testing on a research/investigational basis.*

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**Cytogenetic Laboratory**
**Molecular Genetics Laboratory**
**TTDS Laboratory**
**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.

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**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, FANCI, 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.

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**Complementation Testing** is available on a research basis only. Please call 513-636-5998 for additional details and to arrange testing.