Fanconi Anemia and DNA repair disorders Next Generation Sequencing (NGS) Panel:
Information for Ordering Providers

What is Next Generation Sequencing (NGS)?
NGS is a high-throughput DNA sequencing technology that allows sequencing of multiple regions of the human genome at one time. This enables the simultaneous analysis of many genes known to be associated with a particular phenotype (i.e. gene panels). For some genes, additional analysis for copy number variants may be performed in conjunction with NGS.

Since not all genes associated with a given phenotype/presentation are known or included in the panel, a pathogenic variant will not be identified for every patient. The absence of a pathogenic variant does not exclude a clinical diagnosis.

Testing may identify a genetic variant for which there is currently insufficient evidence to conclude that it is either disease-causing or benign (called a variant of uncertain significance). Such variants cannot be used to alter the clinically established risk of disease.

Why order a NGS panel for my patient?
Phenotypes are often genetically heterogeneous, meaning that the condition is caused by a pathogenic variant(s) in any one of a number of genes. Instead of sequentially testing each of those genes, patients with a particular phenotype should be offered a targeted NGS panel. In some circumstances, it may be more appropriate to test only one gene instead of a panel of genes. In such situations, please contact the laboratory to discuss your patient.

Background
A number of hereditary diseases are characterized by defects in DNA repair including:
Fanconi Anemia (FA) is characterized by physical abnormalities (stature and skeletal limb malformations), bone marrow failure and an increased risk for malignancy. A diagnosis of FA can be established in a patient with increased chromosomal breakage OR a pathogenic variant(s) in a gene known to cause FA. Fanconi anemia (FA) is associated with a number of genes and is most often inherited in an autosomal recessive fashion. FA can also be inherited in an autosomal dominant or X-linked manner.
Ataxia telangiectasia is characterized by progressive cerebellar ataxia, telangiectasias, immunodeficiency and an increased risk for malignancy. It is inherited in an autosomal recessive manner and due to pathogenic variants in ATM.
Bloom Syndrome is characterized by severe prenatal and postnatal growth retardation, sun-sensitive facial erythema and predisposition to multiple cancers. It is inherited in an autosomal recessive manner and is caused by pathogenic variants in BLM.
Nijmegen Breakage Syndrome is characterized by microcephaly, short stature, immunodeficiency and predisposition to cancer. It is inherited in an autosomal recessive manner and caused by pathogenic variants in NBN.
RECQL4-related disorders include Rothmund-Thomson syndrome, Baller-Gerold syndrome and RAPADILINO syndrome. These syndromes all include radial ray defects, skeletal abnormalities, slow growth/short stature and an increased risk for malignancy. They are inherited in an autosomal recessive manner and due to pathogenic variants in RECQL4.

Associated Disorders
Some of the genes on this panel are associated with hereditary cancer including:
Hereditary breast and ovarian cancer: BRCA2, BRIPI, ATM, NBN and RAD51C are associated with an increased risk of breast and/or ovarian cancer, inherited in an autosomal dominant manner.
Familial pancreatic cancer syndrome: PALB2 is associated with an increased risk of pancreatic and breast cancer, inherited in an autosomal dominant manner.

Genetic Laboratory Services
http://www.albertahealthservices.ca/lab/page8667.aspx
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If a pathogenic variant is identified in one of these genes, the patient and/or their family members may be at increased risk for specific cancers. These individuals are eligible for increased cancer screening and/or risk reducing surgeries and therapeutic interventions. In addition, results may influence treatment plans for individuals with cancer. Genetic counselling is recommended for these families.

**Indications for Testing**

Patients presenting with a personal and/or family history suggestive of one of the above syndromes are eligible for testing.

Molecular testing may be considered in situations where cytogenetic testing for FA or chromosomal breakage is not feasible or where cytogenetic testing is positive and identification of the specific variant is valuable.

**Limitations**

In individuals with a haematological malignancy, genetic testing may reveal a variant that is acquired rather than inherited. Confirmatory testing on another tissue (buccal, skin or urine) or a family member may be required.

**Ordering privileges**

This panel may be ordered by Clinical Geneticists, Hematologists, Oncologists and pediatricians on patients with a suspected diagnosis of FA. Presymptomatic testing and carrier testing is restricted to Clinical Geneticists.

The genes included on the Fanconi Anemia NGS panel are:

<table>
<thead>
<tr>
<th>Gene</th>
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<tbody>
<tr>
<td>BRCA2</td>
<td>BRIP1</td>
<td>FANCA</td>
<td>FANCB</td>
<td>FANCC</td>
<td>FANCD2</td>
<td>FANCE</td>
</tr>
<tr>
<td>FANCF</td>
<td>FANCG</td>
<td>FANCI</td>
<td>FANCL</td>
<td>FANCM</td>
<td>PALB2</td>
<td>RAD51C</td>
</tr>
<tr>
<td>SLX4</td>
<td>ERCC4</td>
<td>ATM</td>
<td>BLM</td>
<td>NBN</td>
<td>RECQL4</td>
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**How do I order an NGS panel?**

Discuss the advantages and limitations of testing with your patient (see above). If your patient consents to the testing:

1. Complete the Molecular Diagnostic Laboratory Cancer and Endocrine Next Generation Sequencing Requisition (available at [www.albertahealthservices.ca/lab/page8667.aspx](http://www.albertahealthservices.ca/lab/page8667.aspx)) providing all relevant clinical and family history information. **Incomplete requisitions will not be accepted and will result in test delays.**
2. Provide the fully completed requisition to your patient and **direct them to their local collection lab for a blood draw.**
3. For patients without a valid Alberta PHN, please contact the laboratory genetic counsellor to discuss test availability, billing and sample requirements.

**My patient has a family history of a known pathogenic variant. Is an NGS panel the appropriate test for my patient?**

No. Once a pathogenic variant has been identified in the family it is best to begin testing by looking for the variant that has already been identified in the family.
Methods
Genomic DNA is sequenced on an NGS instrument. Analysis includes the coding region of the gene, including 15bp of intronic/coding boundaries. If a clinically relevant variant does not meet the validation requirements it is confirmed by Sanger sequencing. Additional deletion/duplication testing may be performed by a variety of methods, including, but not limited to: comparative genomic hybridization, NGS-based dosage analysis, multiplex ligation-dependent probe amplification, and quantitative PCR. Confirmation by a secondary method is carried out when necessary. The methods used to generate results are identified on each patient report.

Test Performance
NGS detects nucleotide substitutions, small insertions and deletions and copy number variants. This test is expected to detect >95% of variants in the coding regions of the tested genes.

When can I expect results?
Results may take up to 4 months.

Can testing be expedited to facilitate medical management of a patient?
Expedited testing (~1 month from the time the sample is received) is available if required for immediate surgical or therapeutic management. Please provide details on the requisition form regarding the reason for expedited testing as well as a target date for results.

How are results reported?
Results are sent to the ordering provider. For some tests, results will not be sent to ‘copy to’ physicians by the laboratory, but may be obtained by contacting the ordering health care provider.

What Types of Results Can I Expect?

<table>
<thead>
<tr>
<th>Type of NGS result</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>Pathogenic Variant</td>
<td>A variant has been identified that is disease-causing.</td>
</tr>
<tr>
<td>Likely Pathogenic Variant</td>
<td>A variant has been identified and there is significant but not conclusive evidence that the variant is disease-causing. As per the ACMG guidelines, this denotes a &gt;90% certainty of pathogenicity.</td>
</tr>
<tr>
<td>Variant of Uncertain Significance</td>
<td>A variant has been identified and there is not sufficient evidence to classify the variant as pathogenic/likely pathogenic or benign/likely benign.</td>
</tr>
<tr>
<td>No Pathogenic Variant (Uninformative)</td>
<td>No variants of clinical or uncertain significance were detected. This is an uninformative result and no explanation has been identified for the patient’s phenotype. There may be other genes or variants not assessed by the current NGS panel associated with the patient’s phenotype. A genetic condition or genetic component to the phenotype has not been excluded.</td>
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NOTE: Benign, or likely benign variants (variants known not to be disease-causing) are not reported.

My patient has a variant. What are the next steps?
Your patient should be managed based on their diagnosis and clinical presentation. If your patient has a pathogenic variant or a likely pathogenic variant, genetic counselling may be indicated to discuss the implications for other family members. If your patient has a variant of uncertain significance, a referral to Clinical Genetics may aid in the assessment of the variant.
My patient’s results are uninformative. What are the next steps?
A referral to Clinical Genetics may still be appropriate for your patient if they have a significant family history suggestive of Fanconi anemia or a disorder of DNA repair and/or desire additional counselling regarding their results.

I have questions about NGS Panels. Who do I talk to?
Health care providers can contact the Genetic Laboratory Services Genetic Counsellors at 780-407-1015.

Requisition forms, contact information and other resources can be found at:
http://www.albertahealthservices.ca/lab/page8667.aspx