Endocrine Disorders Next Generation Sequencing (NGS) Panel:
Information for Ordering Providers

What is Next Generation Sequencing (NGS)?
NGS is a high-throughput DNA sequencing technology. Similar to Sanger sequencing, NGS tracks the addition of labeled nucleotides as the DNA chain is copied. Unlike Sanger sequencing where a single DNA fragment is sequenced, NGS allows sequencing of millions of fragments (massively parallel sequencing) at one time, allowing for the generation of more sequence data.

**Advantages:** NGS allows for simultaneous analysis of many genes known to be associated with a particular phenotype; thereby allowing for the implementation of panel testing rather than single gene testing.

**Limitations:** NGS cannot detect large deletions or duplications, triplet repeat expansions and typically includes only the coding regions (exons) of the target genes. For some genes, deletion and duplication testing will 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 diagnosis or indicate low risk of a hereditary cancer syndrome.

Testing may identify a genetic variant for which there is currently insufficient evidence to conclude that it is disease causing or of no effect. 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. If you believe this to be the case for your patient, contact the laboratory to discuss.

Individuals who carry a pathogenic variant in a hereditary cancer gene have an increased risk of certain cancers compared to the general population. 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.

Background
The endocrine disorders represented on this panel are characterized by dysfunction or tumours of one or more endocrine gland. The genes analyzed on this panel encompass several different endocrine syndromes.

The syndromes represented on this panel are inherited in an autosomal dominant manner. The actual risk to develop gland dysfunction or an endocrine tumour is dependent upon the gene involved, the penetrance and the parent of origin.

Indications for testing
Patients presenting with familial hyperparathyroidism or features consistent with MEN are eligible for testing.

Ordering privileges
This panel may be ordered by Clinical Geneticists or Endocrinologists. Presymptomatic testing for a known mutation in the family is restricted to Clinical Geneticists.

Genetic Laboratory Services
http://www.albertahealthservices.ca/lab/page8667.aspx

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The genes included on the Endocrine Disorders NGS panel are:

<table>
<thead>
<tr>
<th>AIP*</th>
<th>CASR*</th>
<th>CDC73*</th>
<th>CDKN1B*</th>
<th>MEN1*</th>
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<tbody>
<tr>
<td>PRKAR1A*</td>
<td>RET</td>
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</table>

*deletion and duplication testing is performed for these genes

How do I order an NGS panel?
Discuss the advantages and limitations of testing with your patient (see above). If the patient consents to the testing:

1. Complete and sign the Edmonton or Calgary Molecular Diagnostic Laboratory 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 delay testing and results.**
2. Indicate the Endocrine Disorders Panel on the requisition in the ‘Hereditary Cancer’ section on the requisition
3. Provide the fully completed requisition to your patient and direct them to their local collection lab for a blood draw.
4. 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.

In addition to the hereditary endocrine disorders caused by variants in the genes on this panel, this panel can also detect the following associated disorders:

- **Hirschsprung disease** is characterised by the complete absence or neuronal ganglion cells from a portion of the intestinal tract. Affected individuals are often diagnosed in infancy, however, some are not diagnosed until childhood or adulthood. Hirschsprung disease can occur as part of a syndrome or as an isolated condition. Pathogenic variants in RET are associated with both syndromic and non-syndromic presentations.

- **Congenital central hypoventilation syndrome** is a disorder of respiratory and autonomic regulation. The classic presentation (apparent hypoventilation, autonomic nervous system dysregulation and variable anomalies / tumors of neural crest-derived structures) is in newborns but a milder later onset can present later in life. Most affected individuals have a pathogenic variant in PHOX2B, however, variants have also been reported in RET.

If a pathogenic variant is identified in one of these genes, the patient and / or their family members may be at increased risk to have a child with one of these disorders. Genetic Counselling is recommended in for these families.

Methods
Genomic DNA is sequenced on an NGS instrument. Sanger sequencing is used to confirm all variants with clinical or uncertain significance and to analyze regions with <90% technical sensitivity by NGS. Deletion/duplication testing is performed for those genes indicated (see above) at single exon-level resolution. A variety of methods may be used for this analysis, 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. All methods used to generate results are identified on each patient report.
Test Performance
NGS detects nucleotide substitutions and small insertions and deletions, and this test is expected to detect >95% of variants in the coding regions of the tested genes. Deletion/Duplication testing detects exon level deletions and duplications and is expected to detect >95% of large deletions/duplications in 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.

How are results reported?
Results are sent to the ordering provider. Results are not available in Netcare. For some tests, results will not be sent to ‘copy to’ physicians by the laboratory but can 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>
</tr>
</thead>
<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.</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</td>
</tr>
<tr>
<td>Uninformative/Normal</td>
<td>No variants of clinical or uncertain significance were detected. An explanation has not been identified for the patient’s phenotype. There may be other genes or mutations not assessed by the current NGS panel associated with the patient’s phenotype. An uninformative result does not exclude a genetic condition or genetic component to the phenotype.</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?
If your patient has a pathogenic variant, a likely pathogenic variant or a variant of unknown significance, a referral to Clinical Genetics is recommended.

My patient’s results are negative. 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 hereditary cancer 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 Calgary Genetic Laboratory Services Genetic Counsellors at 403-955-3097.

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