Appendix A. CCMG Practice Guideline: Laboratory Guidelines for Next-Generation Sequencing, List of Recommendations

# Recommendations for Technical Procedures: Target Regions, Template Preparation and Sequence Generation:

## *Target Enrichment and Library Construction:*

**Recommendation 1:** The NGS assay target region shall be defined; those areas that do not meet assay quality metrics shall be tested using an alternate method or removed from the reported target region.

**Recommendation 2:** Assay quality metrics for successful target enrichment and library construction shall be defined during validation for the specific method and intended use.

## *Sequence Generation:*

**Recommendation 3:** Sequence generation data quality metrics shall be defined for each specific application.

## *Sequence Alignment*

**Recommendation 4:** Sequence alignment quality metrics shall be identified, and thresholds defined for acceptable alignment.

**Recommendation 5:** Consideration shall be given to reducing the risk of incorrect variant calls by appropriate investigation of genomic regions of known homology, such as pseudogenes.

## *Variant Calling:*

**Recommendation 6:** Bioinformatic tools shall be assessed for the ability to reliably detect clinically relevant variant types.

## *Variant Annotation and Interpretation:*

**Recommendation 7:** Laboratories shall use published guidelines for variant classification and interpretation.

## *Analysis of Variant Allele Frequency:*

**Recommendation 8:** For inherited disorders, laboratories shall define the variant allele frequency range corresponding to the heterozygous and homozygous state.

**Recommendation 9:** For acquired cancer or disorders of the mitochondrial genome, laboratories shall define the lower limit of variant allele frequency detection.

**Recommendation 10:** For acquired cancer or disorders of the mitochondrial genome, laboratories shall define the precision of the assay across the clinically relevant range of expected variant allele frequencies.

## *Data Storage:*

**Recommendation 11:** Laboratories shall retain variant call files (VCF) analogous to other data interpreted to generate the final clinical report. Where no local retention standards exist, the VCF shall be retained for at least two years. Strong consideration should be given to retaining some form of the raw data for a defined period of time.

**Recommendation 12:** Laboratories shall ensure data storage (including cloud storage if used) complies with Canadian federal and provincial privacy legislation.

# Recommendations for Test Validation or Verification:

## *General Issues for Validation or Verification:*

**Recommendation 13:** Validation or verification of NGS assays shall encompass the complete end-to-end process, including the wet-laboratory steps and data analysis pipeline.

**Recommendation 14:** Validation or verification is required when modifying a previously validated NGS assay, and should be appropriate to the extent of the modification.

## *Estimating Analytical Sensitivity and Specificity:*

**Recommendation 15:** Validation of large panel or genome-wide NGS assays shall include at least 60 variants, including at least 10 variants of each specific variant type to be detected by the clinical assay.

**Recommendation 16:** Validation of genome-wide NGS assays should include the use of well-characterized samples for which consensus variants are known.

**Recommendation 17:** The minimum read depth required for a desired sensitivity should be established for each assay.

# Recommendations for Ongoing Quality Assurance:

## *Assay Controls:*

**Recommendation 18:** All NGS assays should use appropriate measures to assess for potential contamination.

**Recommendation 19:** Sensitivity controls shall be included to ensure the lower limit of detection is maintained, as applicable.

## *Bioinformatics Ongoing Quality Assurance:*

**Recommendation 20:** Laboratories shall establish a procedure to monitor software versions or updates.

**Recommendation 21:** Reference sequences and databases used should periodically be reviewed to ensure appropriate versions are in use.

## *Ongoing Evaluation and Updating of NGS assays:*

**Recommendation 23:** Laboratories shall define procedures for periodic evaluation of the clinical utility of each targeted NGS assay.

**Recommendation 24:** If changes are made to the genes analyzed in an NGS assay, laboratories shall communicate these gene changes to clinical stakeholders.

**Recommendation 25:** Laboratories shall only review the classification of a previously reported variant at the request of a health care provider acting on behalf of the patient.

# Tests and Clinical Issues:

## *Incidental and Secondary Findings:*

**Recommendation 26:** Laboratories shall define and disseminate policies regarding identification and reporting of incidental or secondary findings.