CANADIAN BIOMARKER QUALITY ASSURANCE
PROGRAMME CANADIEN D'ASSURANCE
QUALITÉ DES BIOMARQUEURS

Canadian Biomarker Quality Assurance Non-small Cell Lung Cancer NGS Scheme (Ontario), I2IS Grant
March 19, 2025

Background

Ontario clinical laboratories are performing routine testing of variants causing Non-small Cell Lung Cancer (NSLCL) by Next Generation Sequencing (NGS) on DNA and RNA extracted from formalin fixed, paraffin embedded tissue. A scheme for Ontario labs performing NSCLC NGS testing was organized as part of an OICR-funded I2IS grant project led by Dr. Harriet Feilotter. The educational NSCLC NGS testing proficiency testing administered by CBQA was designed to evaluate performance of NGS testing of clinically relevant (Tier I) variants, relevant to targeted drug therapies for NSCLC. Assessment included genotyping, interpretation and details of test methods on submitted reports.

Participating Laboratories

Enrollment was limited to Ontario clinical laboratories performing NSCLC testing using NGS. A total of five laboratories from Ontario enrolled, with all five laboratories submitting test results in the form of anonymized reports.

Samples

- Four cases were sent to participating laboratories, and the samples had been pre-tested on both Illumina NGS and ThermoFisher NGS platforms
- For each of the 4 cases, labs received 3 FFPE sections of 7 microns each, which were prepared on uncoated slides and air dried

Expected Results

Expected genotype results for clinically relevant variants (Tier I) for each of the 4 cases are shown in the table below.

Case Identifier	Expected Genotype – Tier I Variants
Case 1	EGFR c.2235_2249del p.(Glu746_Ala750del)
Case 2	No variants in EGFR, KRAS, or MET
Case 3	Fusion MET Exon 13::MET Exon 15 (RNA); MET c.3082G>T p.(?) (DNA)
Case 4	KRAS c.34G>T p.(Gly12Cys)

Scheme Results

All five enrolled laboratories submitted anonymized reports for the scheme results. NGS methods used amplicon-based NGS library preparation and included ThermoFisher Oncomine Comprehensive Assay v3 with Oncomine Variant Annotator in Ion Reporter (3 labs) and Oncomine Precision Assay on Ion Torrent Genexus Analysis software (2 labs). All five laboratories tested both extracted DNA and RNA by NGS. No test failures were noted by any laboratories.

Regarding Genotyping results, all five laboratories correctly identified the expected Tier I genotypes for all cases shown in the table above.

The most significant Genotyping finding was related to nomenclature for Case 3 which had a *MET* gene fusion with deletion of *MET* Exon 14. For Case 3, all five laboratories corrected reported the *MET* Exon 13::*MET* Exon 15 fusion from RNA testing and the related change *MET* c.3082G>T from DNA testing, however four of five labs called this change MET p.(Asp1028Tyr) at the protein level. As the amino acid position 1028 would begin within the coding region of exon 14, which was demonstrated by RNA testing to be deleted by all labs, the correct nomenclature would be p.(?) rather than noting this as a missense mutation.

Other minor Genotyping findings included not using a variant classification system in reporting, and other minor nomenclature deviations from HGVS recommendations.

Regarding Interpretation, and Methods and Clerical sections, no laboratories had any deductions for any cases. This likely reflects that NGS testing for NSCLC for clinically significant genes has been in place within Ontario labs for some time, with a well established interpretation and reporting process in provincial laboratories.