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

Canadian Biomarker Quality Assurance (CBQA) FGFR2 Gene Fusion Scheme (for Cholangiocarcinoma)
September 9, 2024

Background

Canadian clinical laboratories are performing routine testing of gene fusions by Next Generation Sequencing (NGS) on RNA extracted from formalin fixed, paraffin embedded tissue. The educational *FGFR2* fusion testing proficiency testing administered by CBQA was designed to evaluate performance of NGS testing of *FGFR2* fusions in Canadian laboratories, relevant to targeted drug therapies for *FGFR2* fusions in cholangiocarcinoma. The scheme was educational for participating laboratories and not formally marked.

Participating Laboratories

A total of 11 Canadian laboratories enrolled, with 8 laboratories submitting test results on the CBQA website by the scheme deadline. In addition, 6 of the 8 labs uploaded example reports for *FGFR2* testing (an optional activity). The 8 participating laboratories were from four provinces (Nova Scotia, Ontario, Saskatchewan, Alberta).

Samples

- Four FFPE samples were chosen to send to participating laboratories
- For each of the 4 samples, labs received 3 FFPE sections of 7 microns each, which were prepared on uncoated slides and air dried
- A stained H&E slide marking the area of the tumor was also sent to each lab for use in macrodissection
- 2 samples included the most frequent *FGFR2* fusion gene partner *BICC1*
- As *FGFR2* also has many rare gene fusion partners, as seen in more than 50% of *FGFR2*-fusion positive patients, 2 samples with rare fusion partners *SORBS3* and *STAU2* were also used

Expected Results

Expected results for each of the 4 samples are shown in the table below.

Block ID	Material Type	Cancer Type	Expected Gene Fusion	BSI ID	Block ID	Subject ID
1	FFPE	CCA	*FGFR2-SORBS3*	AAA172224 0000	F29563.3Da	D22456
2	FFPE	CCA	*FGFR2-BICC1*	AAA072899 0000	F58152.1b	D33441
3	FFPE	CCA	*FGFR2-BICC1*	AAA072901 0000	F58156.1b	D33445
4	FFPE	CCA	*FGFR2-STAU2*	AAA072895 0000	F58177.Bc	D33452

Laboratory Methods

Laboratories used various NGS library preparation methods and sequencing platforms. Library preparation methods are particularly relevant in *FGFR2* fusion testing due to the known frequency of rare fusion partner genes in cholangiocarcinoma, and the need to detect fusions with both common and rare gene partners. The NGS library methods used by participating laboratories included target-specific library preparation methods (such as amplicon libraries; Group A) or target-agnostic library preparation methods (such as anchored multiplex PCR or hybridization capture libraries; Group B). Details of NGS methods are listed below.

- Group A: Target-specific library preparation methods:
  - ThermoFisher (TF) Oncomine Precision Assay GX on TF Genexus sequencer (amplicon): 2 labs
  - TF Oncomine Comprehensive Plus Assay on TF Ion Torrent S5 sequencer (amplicon): 3 labs
  - Illumina AmpliSeq Focus Panel on MiSeq sequencer (amplicon): 1 lab

- Group B: Target-agnostic library preparation methods:
  - Archer DX FusionPlex Lung on TF Genexus sequencer (anchored multiplex PCR): 1 lab
  - Illumina Custom Assay on NextSeq sequencer (hybrization capture): 1 lab

Scheme Results

Group	Method	*FGFR2-SORBS3* Fusion	*FGFR2-BICC1* Fusion	*FGFR2-BICC1* Fusion	*FGFR2-STAU2* Fusion
A	Oncomine Precision Assay	Not detected	Detected	Detected	Not detected
A	Oncomine Precision Assay	Not detected	Detected	Detected	Exp. Imbalance[1]
A	OCA Plus	Detected	Detected	Detected	Exp. Imbalance[1]
A	OCA Plus	Detected	Detected	Detected	Not detected
A	OCA Plus	Not detected[2]	Detected	Detected	Not detected
A	AmpliSeq Focus Assay	Not detected	Detected	Detected	Testing failed[3]
B	Archer DX FusionPlex Lung	Detected	Detected	Detected	Detected
B	Custom Hyb Capture	Detected	Detected	Detected	Not detected[4]

- 8 laboratories submitted results
- Only the Archer DX FusionPlex Lung detected all four fusion targets
- Three labs detected 3 out of 4 fusions, these labs used either the OCA Plus assay or a custom hybridization capture library method
- [1]Two labs using amplicon panels (OCA Plus or Oncomine Precision) did not directly detect the *FGFR2-STAU2* fusion but did detect a potential *FGFR2* fusion by an expression imbalance assay. The expression imbalance assay does not identify the specific gene fusion partner, and additional testing using orthogonal methods is required to determine the fusion gene partner
- [2]One lab using OCA Plus did not detect the *FGFR2-SORBS3* fusion, although 2 other labs using the same panel and same analysis (Ion Reporter) did detect the fusion. This finding suggests differences in application of the analysis (such as cutoff thresholds) or due to different analysis versions
- [3]One sample failed testing on AmpliSeq Focus assay
- [4]Testing did detect the *FGFR2-STAU2* fusion, but at a level below the limit of detection/ laboratory established threshold for reliable reporting

Interpretation and Reporting

Six laboratories submitted example clinical reports (an optional activity). For review of the submitted reports, report elements suggested by three published guidelines and consensus statements relevant to fusion gene reporting were used (see References). A summary of the expected report elements for each of the six labs are shown below. In general, most report elements were present in all reports, with gaps observed in labs not using a variant classification tier system (3 labs), not including a reference sequence relevant to gene fusions (2 labs), or not including test limitations (1 lab).

Guideline consensus statements on aspects of reporting[1,2,3]	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
Variants reported using HGVS recommendations for fusions i.e. exons involved in fusion are listed, gene partners separated by ::	Y	Y	Y	Y	Y	Y
Variants classified by a tier system	N	Y	N	Y	Y	N
Reference sequence included i.e. NM number	N	Y	Y	N	Y	Y
Relevant statement of test methods i.e. includes fusion detection information	Y	Y	Y	Y	Y	Y
Region covered by test, may also reference website or data sheet	Y	Y	Y	Y	Y	Y
Types of variants detectable by the test	Y	Y	Y	Y	Y	Y
Statement of performance metrics, especially lower limit of detection and minimum seq depth required i.e. minimum fusion reads	Y	Y	Y	Y	Y	Y
Any test limitations e.g. low tumor content	Y	Y	Y	N	Y	Y
Interpretive statement to put variant in clinical context	Y	Y	Y	Y	Y	Y

References

1. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, Tsimberidou AM, Vnencak-Jones CL, Wolff DJ, Younes A, Nikiforova MN. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23. doi: 10.1016/j.jmoldx.2016.10.002.
2. Stockley TL, Lo B, Box A, Gomez Corredor A, DeCoteau J, Desmeules P, Feilotter H, Grafodatskaya D, Hawkins C, Huang WY, Izevbaye I, Lepine G, Papadakis AI, Park PC, Sheffield BS, Tran-Thanh D, Yip S, Sound Tsao M. Consensus Recommendations to Optimize the Detection and Reporting of *NTRK* Gene Fusions by RNA-Based Next-Generation Sequencing. Curr Oncol. 2023 Mar 31;30(4):3989-3997. doi: 10.3390/curroncol30040302.
3. Hume S, Nelson TN, Speevak M, McCready E, Agatep R, Feilotter H, Parboosingh J, Stavropoulos DJ, Taylor S, Stockley TL; Canadian College of Medical Geneticists (CCMG). CCMG practice guideline: laboratory guidelines for next-generation sequencing. J Med Genet. 2019 Dec;56(12):792-800. doi: 10.1136/jmedgenet-2019-106152.