building towards
cIQc
canadian Immunohistochemistry Quality control

Assessors' report for cIQc Run 37: BRAF V600E (April 2014)

Assessors: B Gilks, R Wolber, K Ung, P Tavassoli, J Garratt and J Won (recorder)

Assessment performed on Tuesday, July 29, 2014, at Lions Gate Hospital, North Vancouver

Background

BRAF V600E somatic mutations reportedly account for approximately 70% of cases of loss of MLH1 protein expression in colorectal carcinomas and, when present, essentially exclude concurrent MLH1 Lynch-associated germline mutations. BRAF mutation is likewise exclusive of concurrent K-ras mutation and, like K-ras mutations, precludes a clinical response to EGFR inhibitors in colonic adenocarcinoma. BRAF V600E mutation in the absence of MLH1 deletion selects a subset of colorectal carcinomas with an aggressive clinical course. Therefore, identification of BRAF V600E mutation is of both therapeutic and prognostic significance. Testing for BRAF mutation does not appear to have a clinical role in endometrial cancer.

Overview

The purpose of the challenge was to ensure optimized IHC protocols in laboratories and to establish "best practice" in BRAF V600E IHC. Participating laboratories were asked to stain a colorectal carcinoma tissue microarray enriched for MLH1-deficient cases that have been subjected to BRAF V600E mutational analysis by PCR in the laboratory of Dr. Charles Haynes (Professor in the Department of Chemical & Biological Engineering at UBC) in the Michael Smith Laboratories. All cores were taken from colorectal resections (and one cytology effusion) from a single institution. Available slides from all other participating labs were blindly reviewed by cIQc assessors. Independent review led to infrequent alteration of original self-reported results due to a score being deemed as discordant between self-assessment and final cIQc review then reclassified based on cIQc assessor consensus.

General Observations

Cores 4 and 11 were excluded from all analyses due to high dropout. As indicated to participants in the challenge invitation letter, Core 19 could serve as an excellent weak positive on-slide control for IHC. **We strongly recommend the use of a weak positive on-slide control for BRAF V600E immunostaining!** Furthermore, Core 24 was taken from a case possessing the V600R mutation and was correctly called negative by IHC, an indication of the specificity of the VE1 clone.

Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Although perfect concordance with molecular results there was weak signal making interpretation challenging in some cases.
111	Suboptimal**	Although only two false positive results, there was high background and weak signal (low signal to background ratio). However, it should be noted that IHC optimization is still in progress at this lab.

114	Adequate	Although perfect concordance with molecular results there was weak signal making interpretation challenging in some cases.
116	Adequate	Although perfect concordance with molecular results there was weak signal making interpretation challenging in some cases.
123	Adequate (borderline)	Although perfect concordance there was increased background staining, including unusual nuclear staining. Background staining made it difficult to distinguish between weak positive and negative cases (e.g. Cores 8 and 10, which show weak cytoplasmic positivity but are mutation negative).
175	Adequate	There was perfect concordance and strong staining of the positive cases. Although the self-assessment included many equivocal cases, these were considered negative by the review panel. There was some increased background (e.g. Core 1).
189	Adequate (borderline)	There was one false positive result (Core 15). There was also high background and weak signal (low signal to background ratio), with positive cores showing weak staining.
191	Adequate	Although perfect concordance with molecular results there was weak signal making interpretation challenging in some cases.
193	Optimal	This slide showed clearly superior staining! Although Core 4 was a "false positive" based on self-assessment, there was no tumour in that core upon review. The signal to background ratio was significantly better for this lab's staining than for any of the other labs.
202	Suboptimal	There were three false positives, as a result of high background staining, with weak cytoplasmic immunoreactivity seen in mutation negative cases. True positive cores showed relatively weak staining (low signal to background ratio).

*Based on cIQc assessor consensus
**Lab 111 is still in the process of optimizing the antibody.

The overall results of cIQc Run 37 are promising in that a high degree of sensitivity and specificity was demonstrated. Staining of colonic adenocarcinomas for mutant BRAF is acknowledged to be very challenging, as the staining of positive cases is less intense than is seen in melanoma, but the results of this challenge indicate that it is technically possible. An interesting observation was that, although most labs had adequate staining and were able to distinguish correctly between tumours with and without BRAF mutations, Lab 193 had clearly superior staining compared to all other labs, in that the positive cores were more intensely positive, with no significant increase in background staining. Lab 193 was the only lab that used an amplification step in their staining protocol (Supplementary Table S2).

The BRAF V600E stain was quite specific for tumour cells with no staining of adjacent normal tissue, making for a good internal negative control. Most aberrant/background staining did not interfere with interpretation (e.g. staining of normal smooth muscle cells). Non-specific nuclear staining in tumour cells was observed, particularly in well differentiated colon cancers (e.g. Core 1; Figure 1A). Staining of the whole section of a case that had both a well differentiated and poorly differentiated component showed that only the well differentiated component had nuclear staining, supporting the conclusion that when seeing positive staining of tumour nuclei caution in interpretation should be applied.

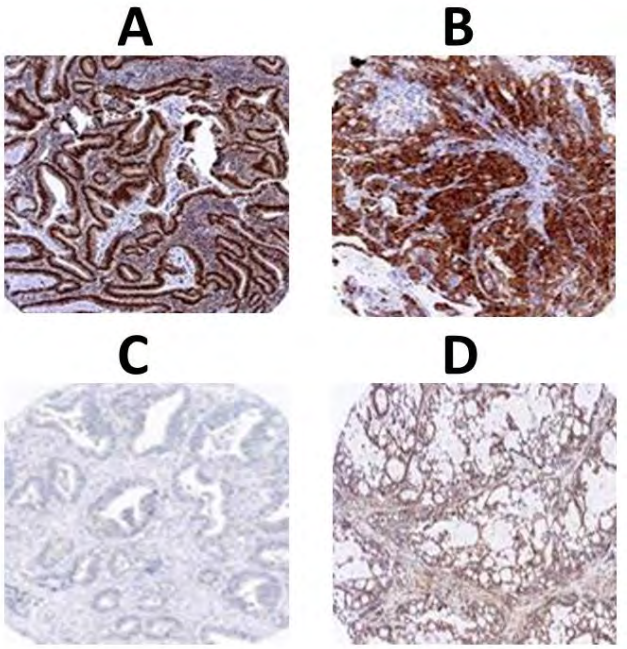

FIGURE 1. Representative images of BRAF V600E IHC. (**A**) Intense background nuclear staining in Core 1 by Lab 123. (**B**) Observed optimal staining in Core 9 by Lab 193. (**C**) Generally weak staining observed in Core 19 by several participating laboratories. (**D**) Suboptimal staining in Core 10 due to low signal to background ratio (Lab 202).

The corrected Garrattogram for BRAF V600E IHC results is provided in Supplementary Figure 1. Supplementary Table 1 summarizes kappa agreement values, sensitivity and specificity of each participating laboratory based on self-assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Your regular participation in cIQc is greatly appreciated and we look forward to continually working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Figure S1. Corrected Garrattogram after cIQc BRAF V600E IHC assessment.

Lab/Core	101	111	114	116	123	175	189	191	193	202	R1	BRAF	MT Freq. (%)
1	N	E	N	N	E	E	E	E	N	E	N	WT	0
2	P	P	P	P	P	P	P	P	P	P	P	V600E	19
3	N	N	N	N	N	N	N	N	N	N	N	WT	0
4	U	U	U	U	P	U	U	P	N	U	P	V600E	17
5	E	P	N	N	N	E	E	U	N	E	N	WT	0
6	P	P	P	P	P	P	P	P	P	P	P	V600E	5
7	P	P	P	P	P	P	E	P	P	P	P	V600E	12
8	N	E	N	N	N	E	N	N	N	P	N	WT	0
9	P	P	P	P	P	P	P	P	P	P	P	V600E	9
10	N	N	N	N	E	N	N	E	N	P	N	WT	0
11	U	U	U	U	U	U	U	U	U	U	N	WT	0
12	P	P	P	P	P	P	P	P	P	P	P	V600E	8
13	N	N	N	N	N	N	N	N	N	N	N	WT	0
14	P	P	P	P	P	P	P	P	P	P	P	V600E	16
15	E	P	N	N	E	E	P	N	N	P	N	WT	0
16	P	P	P	P	P	P	P	P	P	P	P	V600E	20
17	P	P	P	P	E	P	P	P	P	P	P	V600E	8
18	P	P	P	P	P	P	P	P	P	P	P	V600E	13
19	P	P	P	P	P	P	P	P	P	P	P	V600E	27
20	N	N	N	N	N	E	N	N	N	N	N	WT	0
21	P	P	P	P	P	P	P	P	P	P	P	V600E	12
22	N	E	N	N	E	E	E	E	N	E	N	WT	0
23	P	P	P	P	P	P	P	P	P	P	P	V600E	19
24	N	E	N	N	N	E	N	N	N	N	N	V600R	35
25	P	P	P	P	P	P	P	P	P	P	P	V600E	12
26	N	E	N	N	N	N	N	N	N	N	N	WT	0
27	P	P	P	P	P	P	P	P	P	P	P	V600E	25
28	P	P	P	P	P	P	P	P	P	P	P	V600E	47
29	P	P	P	P	P	P	P	P	P	P	P	V600E	14
30	P	P	P	P	E	P	P	P	P	P	P	V600E	14
31	N	N	N	N	N	N	N	N	N	N	N	WT	0
32	P	P	P	P	P	P	P	P	P	P	P	V600E	12
33	P	P	P	P	P	P	P	P	P	P	P	V600E	10
34	P	P	P	P	P	P	P	P	P	P	P	V600E	13
35	N	N	N	N	E	N	N	N	N	E	N	WT	0
36	P	P	P	P	P	P	P	P	P	P	P	V600E	4
37	P	U	P	P	P	P	P	U	P	P	P	V600E	8
38	N	N	N	N	N	N	N	N	N	N	N	WT	0
39	P	P	P	P	P	P	P	P	P	P	P	V600E	15
40	N	N	N	N	E	E	N	N	N	E	N	WT	0
41	N	N	N	N	E	E	N	N	N	N	N	WT	0

N Neg P Pos E Equiv U Unsat

Table S1. BRAF V600E descriptive statistics generated from self-assessments.

Lab ID	Total n	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	41	95.12	39	38/39 (97%)	1	0.94	0.96	1	0.95
111	41	92.68	38	36/38 (95%)	1	0.88	0.92	1	0.89
114	41	95.12	39	39/39 (100%)	1	1	1	1	1
116	41	95.12	39	39/39 (100%)	1	1	1	1	1
123	41	97.56	40	40/40 (100%)	1	1	1	1	1
175	41	95.12	39	39/39 (100%)	1	1	1	1	1
189	41	95.12	39	38/39 (97%)	1	0.94	0.96	1	0.95
191	41	92.68	38	38/38 (100%)	1	1	1	1	1
193	41	97.56	40	39/40 (98%)	0.96	1	1	0.94	0.95
202	41	95.12	39	36/39 (92%)	1	0.81	0.88	1	0.84

Table S2. Reported BRAF V600E staining protocols.

Lab ID	Clone	Dilution	Supplier	Ab Lot #	Ab Incubation Time	Ag Retrieval	Detection	Enhancement
101	VE1	1:50	Spring	131216A	32 minutes	CC1	Optiview	Copper
111	VE1	1/200 (being optimized)	Spring Bioscience	1303180	32 minutes	CC1 - 36 minutes	Ventana Ultraview	Amplified (M)& Copper
114	VE1	1/100	Spring Bio	131216A	16 min	CC1 32min	Optiview Ventana	Copper
116	VE1	RTU	VENTANA	D04837Z	28 M	CC1 64M	OPTIVIEW DAB IHC	NO
123	VE1	1/600	Spring Bioscience	not sure	30 min	Tris-EDTA pH9	Biogenex Super Sensitive Kit	none
175	VE1	Pre-dilute	Ventana	E00169	16 min	CC1 64 mins	Optiview	Copper and amplification
189	VE1	Pre-dilute	Ventana	D04836Z	60 min.	CC1	Optiview DAB Detection Kit	Copper
191	VE1	RTU	Roche	D04836Z	16 min	CC1	optiview	none
193	VE1	1/800	Spring Bio	121220CA	36 min at 37 deg	CC1 40 min	Optiview with amplification kit	no
202	VE1	1/40	Spring	130226C	16 min	Epitope retreival #2 Leica	Leica Refine detection system	none