Assessors' report for cIQc Run 48: BRAF V600E (April 2015)

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Assessment performed on Wednesday, July 15, 2015, 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."

— cIQc Run 37 Summary

Overview

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

Core 32 was excluded from all analyses as this particular sectioning of the array began to cut out of BRAF V600E-positive carcinoma and into a BRAF V600E-negative adenoma. Core 41 was 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.

For any laboratories using an amplification kit, it has been noted that room temperature equilibration of the kit prior to application significantly improves staining results. This observation has been brought forth to Ventana.

Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Threshold for positivity may be too high since many equivocal cases by self-assessment were deemed to be positive by cIQc assessors. Weak positive control (Core 19) on the array was confirmed negative.

111	Adequate	Positive cores generally had weak staining. Use of amplification kit recommended to increase staining intensity.
114	Optimal	
116	Failed	Although good concordance with molecular results there was a general cytoplasmic blush that essentially made all negative cases appear to have equivocal staining. It was noted by assessors that this lab has a particularly long antibody incubation time compared to other laboratory protocols.
123	Failed	There was also high background and weak signal (low signal to background ratio), with most cores possessing some degree of staining. It should be noted that this lab is using BRAF V600E staining for research only, and their pathologists have already communicated their concern of suboptimal staining to cIQc.
160	Optimal	
175	Optimal	There was perfect concordance and strong staining of the positive cases. Heavy nuclear background staining, which may have been responsible for observed cytoplasmic “bleeding”.
189	Adequate	Positive cores generally had weak staining. Use of amplification kit recommended to increase staining intensity.
191	Sub-optimal	Although perfect concordance with molecular results there was strong cytoplasmic background and weak signal in positive cores, making interpretation challenging in some cases. Weak positive control (Core 19) on the array was confirmed negative. Use of amplification kit recommended.
193	Adequate	Generally weaker staining compared to other labs. Weak positive control (Core 19) on the array was determined to have equivocal staining by cIQc assessors.
202	Adequate	Slight background staining, with weak cytoplasmic immunoreactivity seen in mutation negative cases.

215	Optimal	
217	Adequate	Several cores with equivocal staining were confirmed by cIQc assessors. Use of amplification kit recommended to increase staining intensity.

*Based on cIQc assessor consensus

The overall results of cIQc Run 48 continue to demonstrate a high degree of sensitivity and specificity for BRAF V600E IHC. As noted in the run summary of the inaugural BRAF V600E challenge (Run 37), use of an amplification kit appears to significantly improve the intensity of positive staining, with no significant increase in background staining. Since then, several labs have now incorporated use of the amplification kit in their staining protocol. As briefly mentioned above, it has been brought to our attention that room temperature equilibration of the kit prior to application significantly improves staining results.

The revised Garrattogram for BRAF V600E IHC results is provided in Supplementary Figure 1. Supplementary Table 1 lists staining details submitted by each laboratory. 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. Revised Garrattogram after cIQc BRAF V600E IHC assessment.

Lab/ Core	101	111	114	116	123	160	175	189	191	193	202	215	217	R1	BRAF	MT Freq. (%)
1	N	N	N	N	E	N	E	N	N	N	E	N	E	N	WT	0
2	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	19
3	N	N	N	N	E	N	N	N	N	N	N	N	N	N	WT	0
4	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	17
5	N	N	N	N	E	N	N	N	N	N	N	N	N	N	WT	0
6	N	P	P	P	E	P	P	P	E	P	P	P	P	P	V600E	5
7	P	P	P	P	E	P	P	P	E	P	P	P	P	P	V600E	12
8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	WT	0
9	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	9
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	WT	0
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	WT	0
12	P	P	P	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	N	N	N	WT	0
14	P	P	P	P	E	P	P	P	P	P	P	P	P	P	V600E	16
15	N	N	N	N	E	N	N	N	N	N	N	N	E	N	WT	0
16	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	20
17	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	8
18	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	13
19	N	E	P	E	E	P	P	E	N	E	P	P	P	P	V600E	27
20	N	N	N	N	E	N	N	N	N	N	N	N	N	N	WT	0
21	P	P	P	P	E	P	P	P	P	P	P	P	P	P	V600E	12
22	N	N	N	N	N	N	N	N	N	N	N	N	E	N	WT	0
23	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	19
24	N	N	N	N	E	N	N	N	N	N	N	N	E	N	V600R	35
25	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	12
26	N	N	N	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	P	P	P	V600E	25
28	P	P	P	P	P	P	P	P	P	P	P	P	P	P	V600E	47
29	P	P	P	P	E	P	P	P	P	P	P	P	P	P	V600E	14
30	P	E	P	E	E	P	P	P	E	P	P	P	P	P	V600E	14
31	N	N	N	N	N	N	N	N	N	N	N	N	N	N	WT	0
32	N	N	N	N	N	N	E	N	N	N	N	N	E	P	V600E	12
33	P	P	P	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	P	P	P	V600E	13
35	N	N	N	N	E	N	N	N	N	N	N	N	N	N	WT	0
36	P	P	P	E	P	P	P	P	E	P	P	P	P	P	V600E	4
37	P	U	P	E	E	P	P	P	P	P	P	P	P	P	V600E	8
38	N	N	N	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	P	P	P	V600E	15
40	N	N	N	N	E	N	N	N	N	N	N	N	N	N	WT	0
41	U	U	U	N	U	U	U	U	U	N	U	N	U	N	WT	0

Table S1. Reported BRAF V600E staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	64 min	V600E	1:200	Spring Bioscience	131216A	16	OptiView	Y	Y	DAB
111	CC1 (HIER)	48 min	VE1	Predilute	Ventana	F00442	8 min	Optiview	N	Copper	DAB
114	CC1	32min	VE1	1/200	Spring Bio	131216A	16min	Optiview	yes	copper	DAB
116	CC1 (EDTA)	64 min	VE1	RTU	Ventana-Roche	E06579	48 min	Optiview DAB	No	Y	DAB
123	TE-9	120 C, 20 min	original/ homemade	1/500	NA	NA	Overnight	Mach 4	Yes	No	DAB
160	CC1	64 MIN	V600E(VE1)	Predilute	VENTANA	E00169	16 MIN	OPTIVIEW	Y	Y: copper	DAB
175	HIER	64 min	VE1	pre-dilute	Ventana	E06579	16 min	polymer	Y	Y	DAB
189	CC1	64	VE1	pre-dilute	Ventana	NA	16	Optiview	N	N	DAB
191	CC1	64'	VE1	RTU	Roche	ÃŠ00169	16'	optiview	N	N	DAB
193	CC1	40 minutes	VE1	1/800	Biospring	130508K	36 minutes at 37 deg.	Optiview	Yes	no	DAB
202	ER2	30	v600e	1/100	spring	130508j	15	refine detection kit leica	n	n	dab
215	CC1	64	VE1	Predilute	Roche/Ventana	E00169	16	Optiview	Y	N	DAB
217	Ventana ultra CC1	64 min	VE1	predilute	Ventana	D04836Z	16 min	Ventana optiView	n	y	DAB