Assessors' report for cIQc Run 88: BRAF V600E

Assessors: R Wolber, J Garratt and J Won (recorder)

Assessment performed on September 10 and October 19, 2018, 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 74 Summary

Overview

Participating laboratories stained 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 from a single institution.

Core 2 was noted to be a weak positive case but the core condition was variable across labs, with some losing large portions of positive tumour in the core while other labs retained a full core in their section. Variable results for Core 31 were observed possibly due to a fixation issue, so this core should be disregarded. Core 26 was noted to be a good weak positive on-slide control for IHC. Use of a weak positive on-slide control for BRAF V600E immunostaining is strongly recommended!

Participant-specific feedback is summarized below:

Lab ID	IHC Status*	Comment
101	Optimal	
111	Adequate	Weaker staining compared to optimal labs
114	Optimal	
116	Optimal	
138	Optimal	
175	Optimal	
176	--	Technical issue (bubble during Ab incubation?); not interpretable
181	Adequate	Slightly weak staining with minor background
193	Adequate	Generally weak with very weak staining in some cores
199	Adequate	Weak staining
207	Optimal	Slightly weak
217	Optimal	
222	Adequate	Generally weak with very weak staining in some cores
228	Adequate	Weak staining

*Based on cIQc assessment team consensus

Garrattogram after cIQc assessment:

Lab/ Core	101	111	114	116	138	175	181	193	199	207	217	222	228	Mutation Status
1	U	U	U	U	U	U	U	U	U	U	U	U	U	WT
2	P	N	U	U	E	U	U	N	U	U	N	P	N	*V600E*
3	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
4	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
5	P	P	P	P	P	P	P	E	P	P	P	P	P	*V600E*
6	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
7	P	E	P	P	P	P	P	E	P	P	P	P	E	*V600E*
8	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
9	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
10	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
11	E	N	N	N	E	N	N	N	N	N	N	N	N	WT
12	U	N	N	U	N	U	U	U	N	N	N	N	N	WT
13	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
14	N	N	N	N	N	N	N	N	N	N	N	E	N	WT
15	N	N	N	U	N	U	N	N	N	N	N	N	U	WT
16	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
17	N	N	N	N	N	N	N	N	N	N	N	E	N	WT
18	U	U	U	U	U	U	U	U	U	U	U	U	U	*V600E*
19	N	N	N	N	N	N	N	N	N	N	N	E	N	WT
20	N	N	N	N	N	N	N	N	N	N	N	E	N	WT
21	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
22	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
23	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
24	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
25	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
26	P	E	P	P	P	P	E	E	E	P	P	E	E	*V600E*
27	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
28	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
29	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
30	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
31	P	U	U	N	P	U	U	P	P	U	E	P	U	WT
32	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
33	N	N	N	N	N	E	N	N	N	N	N	E	N	WT
34	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
35	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
36	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
37	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
38	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
39	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
40	P	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*

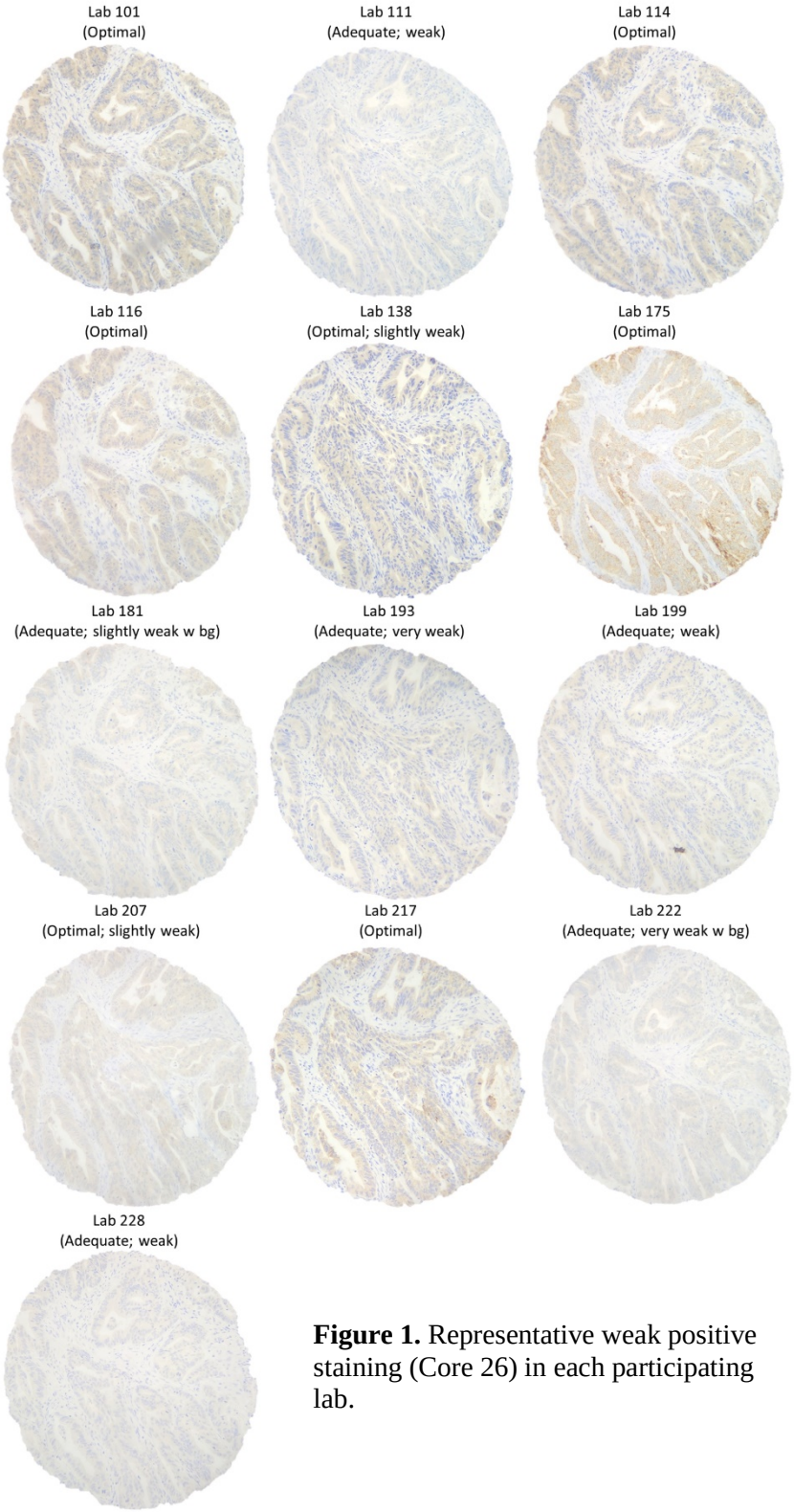

Figure 1. Representative weak positive staining (Core 26) in each participating lab.

BRAF V600E IHC in colorectal carcinoma continues to demonstrate a high degree of sensitivity and specificity. Use of an amplification step (particularly for labs using a Ventana platform) significantly improves the intensity of positive staining, with no significant increase in background staining.

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.

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 No.	Time for Ab Incubation (min)	Detection System	Amplication (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, HIgh PH	1 hour	V600E	1:200	SPRING Bio	150810AE	15 min	DAKO Envision Flex	N	N	DAB
111	CC1	48	VE1	Predilute	Ventana	Y10963	8	Optiview	y	y	DAB
114	High PH	60	VE1	1:200	Spring Bioscience	161116C	15	DAKO OMNIS	Y	N	DAB
116	CC1	80 min	V600E	RTU	Ventana	G03589	44 min	Optiview DAB	N	Y	DAB
138	EDTA HIER	20	VE1	1:100	abcam	GR3217024-5	30	Polymer	Y	N	DAB
175	HIER	64	VE1	Predilute 0.5	Roche	g10381	16	OPTI-DAB (polymer)	Y	Y	DAB
176	CC1	64	VE1	1:200	Spring Bioscience	150810R	32	Optiview	Y	N	DAB
181	HIER pH9	64	VE1	RTU: on-board diln	Roche Ventana	Y16508	8	Optiview HRP Multimer	Y	N	DAB
193	CC1	40 Min.	V600E (VE1)	1/800	Spring Bioscience	150810U	36 Min. @ 37 C	Optiview	Yes	Yes	DAB
207	on line CC1	64 minutes	VE1	Prediluted	Ventana	E01023	32 minutes	Optiview	N	Y	DAB
217	HIER	64	VE-1	Pre-dilute	Roche Ventana	Y10963	16	Optiview	Yes	Yes	DAB
222	Ultra CC1	64	VE1	1:1	Ventana	G05624	36	Optiview DAB	N	Y	Copper
228	HIER using Bond retrieval 2	30	VE1	RTU	Roche (Ventana)	Y10963	15	Leica Bond refine detection system	N	N	DAB