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cIQc
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Assessors' report for cIQc Run 74: BRAF V600E (April 2017)

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Assessment performed on Thursday, July 13, 2017, 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 48 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 from a single institution. Available slides from participating labs were blindly reviewed by cIQc assessors.

Cores 16 and 18 were excluded from all analyses as sectioning of the array began to cut out of BRAF V600E-positive carcinoma or no tumour was present for most laboratories. Core 26 was noted to be a good weak positive on-slide control for IHC. Similarly, Core 2 was also 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. As previously emphasized, **use of a weak positive on-slide control for BRAF V600E immunostaining is strongly recommended!**

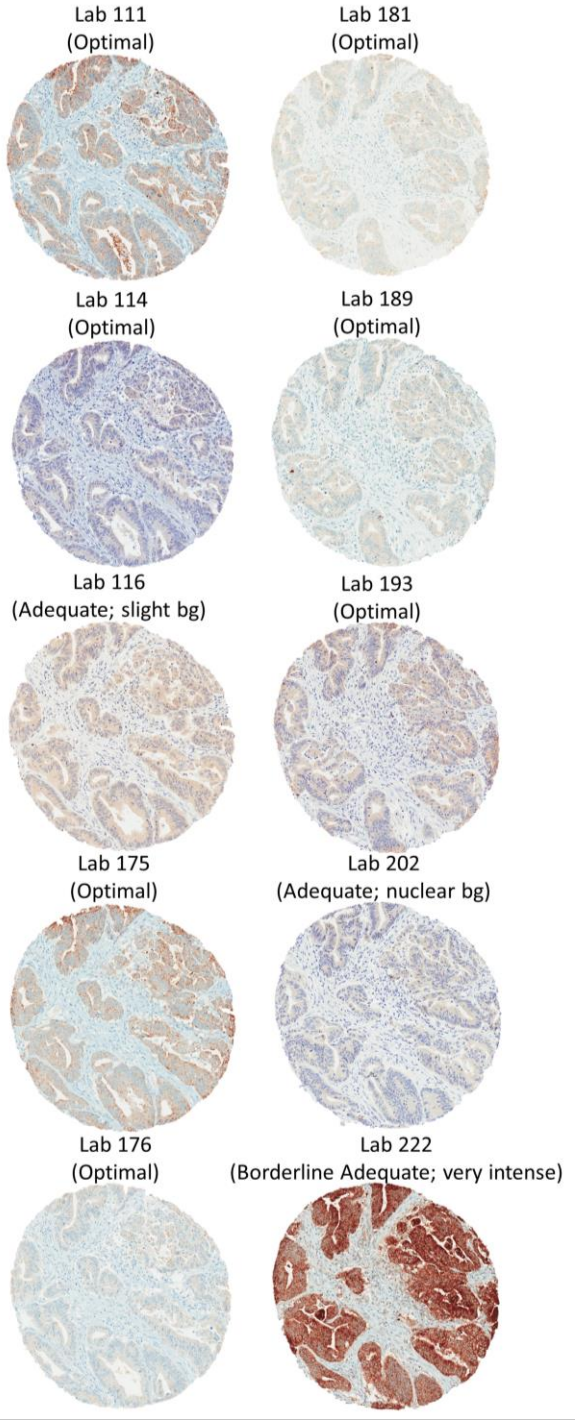
Participant-specific feedback is summarized below:

Lab ID	IHC Status	Comments
111	Optimal	Very nice staining; good counterstain; several "E's" changed to "N" during cIQc review
114	Optimal	
116	Adequate	General blush background (some cores more than others)
175	Optimal	
176	Optimal	Slightly weaker intensity than other labs but still easily interpretable
181	Optimal	Slightly weaker intensity than other labs but still easily interpretable
189	Optimal	Slightly weaker intensity than other labs but still easily interpretable
193	Optimal	
199	--	Slide not available for assessment
202	Adequate	Heavy nuclear background leading a cytoplasmic "bleeding" artifact
217	--	Slide not available for assessment
222	Borderline Adequate	Very intense staining; all equivocal calls during self-assessment confirmed during cIQc assessment

*Based on cIQc assessor consensus

Lab/ Core	111	114	116	175	176	181	189	193	199	202	217	222	Mutation Status
1	U	U	U	U	U	U	U	U	U	U	U	U	WT
2	P	N	P	P	N	N	P	E	E	E	E	P	*V600E*
3	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	*V600E*
5	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
6	N	N	N	N	N	N	N	N	N	N	N	E	WT
7	P	P	P	P	P	P	P	P	P	E	P	P	*V600E*
8	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	E	WT
10	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
11	N	N	N	N	N	N	N	N	N	N	N	E	WT
12	N	N	N	U	N	U	U	U	N	N	N	N	WT
13	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	N	WT
15	N	N	N	N	N	N	N	E	N	N	N	E	WT
16	U	N	N	N	N	N	N	N	N	N	N	E	*V600E*
17	N	N	N	N	N	N	N	N	N	N	N	E	WT
18	U	U	N	U	N	U	U	U	N	U	E	U	*V600E*
19	N	N	N	N	N	N	N	N	E	E	E	E	WT
20	N	N	N	N	N	N	N	N	N	N	E	E	WT
21	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
22	N	N	N	N	N	N	N	N	N	E	N	P	WT
23	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
24	N	N	N	N	N	N	N	E	N	N	E	E	WT
25	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
26	P	P	P	P	E	P	P	P	N	E	P	P	*V600E*
27	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	E	WT
29	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	*V600E*
31	N	N	N	N	N	N	N	U	N	E	N	E	WT
32	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
33	N	N	N	N	N	N	N	N	N	N	N	P	WT
34	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	*V600E*
36	N	N	N	N	N	N	N	N	N	N	N	E	WT
37	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	E	WT
39	N	N	N	N	N	N	N	N	N	N	N	E	WT
40	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.



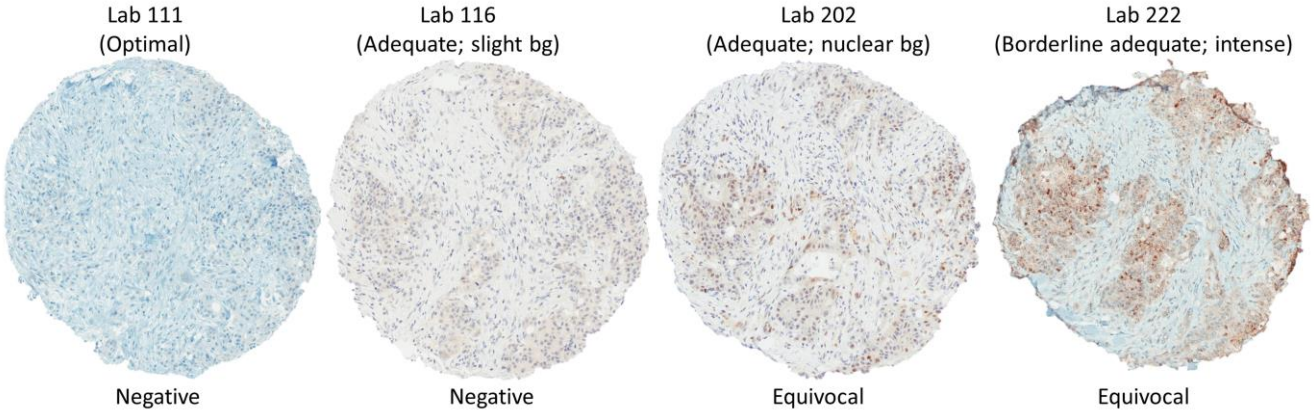


Figure 2. Representative staining of a BRAF wildtype core (Core 19).

Similar to previous BRAF V600E runs using a colorectal carcinoma tissue microarray, a high degree of sensitivity and specificity for BRAF V600E IHC is demonstrated in cIQc Run 74. Use of an amplification kit by many participants appears to significantly improve 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	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
111	HIER	48	VE1	predilute	ventana	G01645	8	optiview	Y	Y	DAB
114	CC1	32	VE1	1/200	Spring Bio	131216A	16	Optiview	Y	Y	DAB
116	CC1	64 min	V600E	RTU	Ventana	E06579	36 min	Optiview DAB	No	Y	DAB
175	HIER	64	VE1	Pre-dilute	Roche	903589	16	OPTI (polymer)	Y	Y	DAB
176	CC1	64	VE1	1:200	Cedarlane/Spring Bio	150810R	32	Optiview	Y	Y	DAB
181	HIER High pH	64	VE1	RTU- on board diln.	Roche Ventana	G05624	8	OV HRP Multimer	Y	N	DAB
193	CC1	40 Min.	V600E (VE1)	1/800	Spring Bioscience	140515A	36 Min. @ 37 deg.	Optiview	Yes	Yes	DAB
199	HIER	20	V600E(VE1)	100	SPRING BIOSCIENCE	150810V	15	BOND Refine (Polymer)	N	N	DAB
202	HIER citrate pH 9.5	30 min	v600e	1/100	Spring	141210LVA	15 MIN	Leica Refine detection kit	no	no	DAB
217	HIER	64	VE-1	pre-dilute	Roche Ventana	F05119	16	Optiview	Yes	Yes	DAB
222	Ultra CC1	64	VE1	1:1	Ventana	G05624	32	Optiview DAB	Y	Y	Copper