Assessors' report for cIQc Run 101: BRAF V600E

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Assessment performed on August 2 and September 18, 2019, 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. Similarly, 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. Identification of BRAF V600E mutation is of both therapeutic and prognostic significance.

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.

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	
114	Optimal	
116	Optimal	
123	Optimal	
160	Optimal	
175	Optimal	
176	Optimal	Slightly weak staining
181	Adequate	Weak staining
189	Adequate	Weak staining
207	Optimal	Nice staining
217	Optimal	Nice staining
228	Adequate	Generally weak staining with very weak staining in some cores
230	Optimal	

*based on CIQC assessor consensus

Garrattogram after cIQc assessment:

Lab ID/ Core	101	114	116	123	160	175	176	181	189	207	217	228	230	Mutation Status
1	U	U	U	U	U	U	U	U	U	U	U	U	U	WT
2	P	U	U	P	U	P	U	U	P	P	U	E	E	*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	E	P	*V600E*
5	P	P	P	P	P	P	P	P	P	P	P	E	P	*V600E*
6	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
7	P	P	P	P	P	P	E	E	P	P	P	E	P	*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	E	P	*V600E*
11	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
12	N	N	U	N	U	N	U	U	U	U	N	N	U	WT
13	P	P	P	P	P	P	P	P	E	P	P	E	P	*V600E*
14	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
15	N	N	N	N	N	N	N	N	N	N	N	N	N	WT
16	P	P	P	P	P	P	P	P	P	P	P	E	P	*V600E*
17	N	N	N	N	E	N	N	N	N	N	N	N	N	WT
18	U	U	U	U	U	U	U	U	U	U	U	U	U	*V600E*
19	N	N	N	N	E	N	N	N	N	E	N	N	N	WT
20	N	N	N	N	N	N	N	N	N	N	N	N	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	P	P	P	P	P	P	E	P	P	P	N	P	*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	E	P	P	P	E	P	*V600E*
31	N	U	U	U	U	U	N	N	N	N	U	N	U	WT
32	P	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	N	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	U	N	N	N	N	N	U	U	U	U	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*

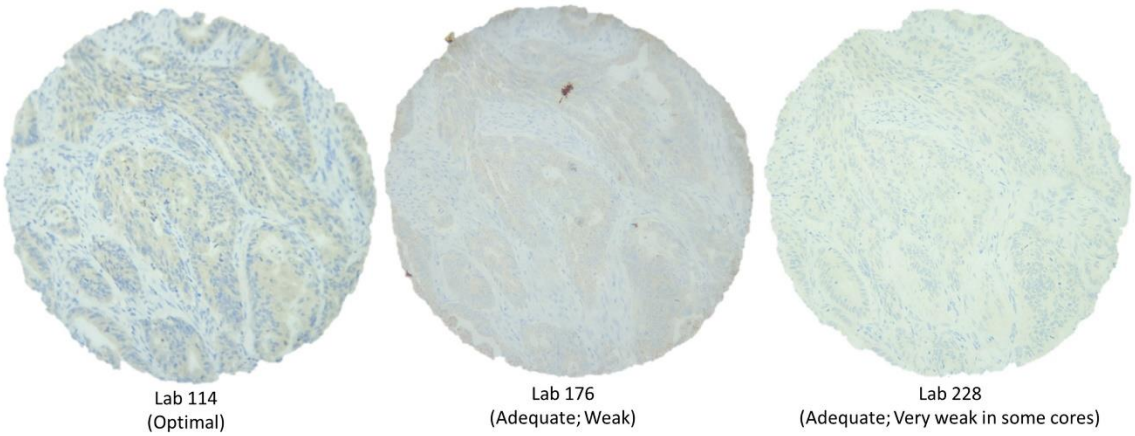

Figure 1. Representative weak positive staining in Core 26.

As noted in previous challenges, BRAF V600E IHC in colorectal carcinoma has 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. However, amp kit variability still remains an issue as illustrated in Figure 2 below of representative staining by two participants using identical protocols with an amp kit.

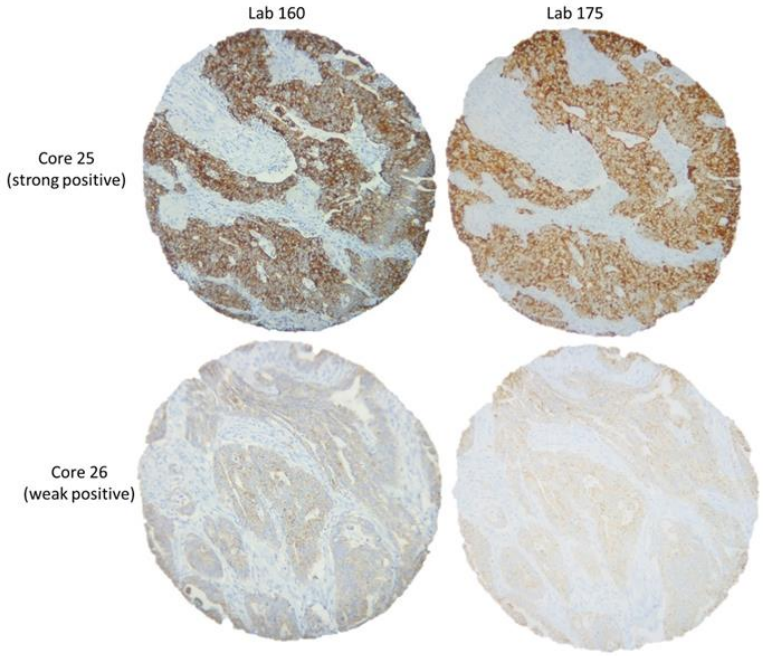

Figure 2. Staining variability of the amp kit as illustrated by comparison of staining by two participants using identical protocols with an amp kit.

Supplementary Table 1 summarizes staining protocols, Supplementary Table 2 summarizes descriptive statistics and Supplementary Table 3 provides the definitions of CIQC IHC Statuses assigned to each participant. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Your participation in CIQC is greatly appreciated and we look forward to continuing to work 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
101	EnV FlexTRS, HIgh PH	60 min	V600E	1:200	SPRING Bio	161116Q	15 min	DAKO Envision Flex	Y	N	DAB
114	Envision Flex TRS, High pH	60	VE1	1:200	Spring Bioscience	161116C	20	Envision FLEX DAKO Omnis	N	y	Envision Flex DAB
116	CC1	80 MIN	VE1	RTU	VENTANA	E17344	44 MIN	OPTIVIEW DAB	N	.	DAB
123	Roche CC1	64	VE1	predilute	Roche	E26550Z	16	Roche OptiView	Y	N	DAB
160	CC1	64 MIN	V600E	PRÃ‰-DILUÃ‰	VENTANA	E17344	16 MIN	OPTIVIEW	Y	Y	DAB
175	HIER	64	VE1	Predilute	Roche	E17344	16	optiview	Y	Y	DAB
176	CC1	64	VE1	1:200	Spring Bioscience	150810R	32	Optiview	y	n	DAB
181	on line	64 minutes	BRAF (VE1)	pre-diluted	Ventana Roche	E17344	8 minutes	OptiView DAB kit	yes	no	DAB
189	CC1	64	VE1	RTU	Ventana	unknown	16	OptiView DAB	N	N	OptiView DAB
207	Envision Flex High PH	30	V600E	1/400	abcam	GR 3235840-23	15	Envision Flex -Magenta	Y	N	MAGENTA
217	HIER	64	VE-1	Predilute	Roche Ventana		16	Optiview	Yes	Yes	DAB
228	HIER using Bond ER2	30	VE1	RTU	VENTANA	E09451	15	BOND REFINE DETECTION KIT	NONE	NONE	DAB
230	HIER	32	VE1	predilute	Roche	E17344	16	Ultraview	Y	N	DAB

Table S2. Descriptive statistics for BRAF V600E IHC based on CIQC assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	40	95	38	38/38 (100%)	1	1	1
114	40	90	36	36/36 (100%)	1	1	1
116	40	85	34	34/34 (100%)	1	1	1
123	40	92.5	37	37/37 (100%)	1	1	1
160	40	87.5	35	35/35 (100%)	1	1	1
175	40	92.5	37	37/37 (100%)	1	1	1
176	40	90	36	36/36 (100%)	1	1	1
181	40	90	36	36/36 (100%)	1	1	1
189	40	90	36	36/36 (100%)	1	1	1
207	40	90	36	36/36 (100%)	1	1	1
217	40	87.5	35	35/35 (100%)	1	1	1
228	40	92.5	37	36/37 (97%)	0.95	1	0.95
230	40	90	36	36/36 (100%)	1	1	1

Table S3. IHC Status definitions.

IHC Status	Definition	cIQc Proficiency Testing Performance
Optimal	The staining was considered of the highest technical quality to allow for accurate readout of the target biomarker.	PASS
Adequate	The staining was considered to be sufficient for the purpose of accurate readout of the target biomarker.	PASS
Sub-optimal	The staining was considered to be of a quality that makes readout of the test challenging, which may lead to inaccurate readout of the target biomarker.	PASS, CONDITIONALLY[1]
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	FAIL[2]

1 – A one-time suboptimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".

1,2 – Please contact the cIQc for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.