Summary Report – Run 117 BRAFV600E

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Overview

Identification of BRAF V600E mutation is of both therapeutic and prognostic significance in colorectal carcinomas. The survey consisted of 40 tissue cores of colorectal carcinomas 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.

No established cut-off for positive versus negative staining was defined by the CPQA-AQCP, and participants were asked to simply score the tumour cells as positive or negative according to current practice at each institution. The scoring system that laboratories were asked to apply was:

- Positive (P) – indicates a V600E mutation is present. Weak, moderate, or strong cytoplasmic positivity in tumour cells (weak positive is clearly positive, but weakly stained).
- Equivocal (E) – borderline, very faint cytoplasmic staining in any tumour cells (use when there is uncertainty whether the cells are really positive or not).
- Negative (N) – Negative – indicates no mutation present. No staining in tumour cells.
- Unsatisfactory (U) – technical problem that makes interpretation impossible, such as core drop off or no tumour cells present

Results

Overall results were excellent for participants. Core 2, a case with BRAFV600E mutation, was noted to be weakly positive but the condition of the core was variable across participants, with some losing large portions of positive tumour. Core 26 was noted to be weakly positive, serving as an ideal on-slide control for IHC. Participant-specific feedback is below:

Lab ID	IHC Status*	Comment
101	Optimal	
111	Optimal	Nice staining
114	Optimal	
123	Adequate	Several equivocal cores due to speckling from the amplification step; confirmed unexplained false-negative in Core 30
149	Optimal	Several portions of cores physically scrapped in the first three columns of the TMA; still interpretable
160	--	Slide not available at the time of assessment
175	Optimal	Nice staining
176	Optimal	Slightly weak
207	Optimal	
217	--	Slide not available at the time of assessment
228	Adequate	Weak staining
230	Optimal	Slightly weak

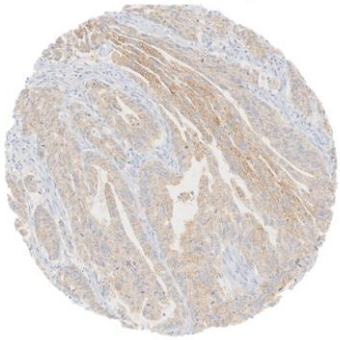
*based on CPQA assessor consensus

Garrattogram after CPQA assessment:

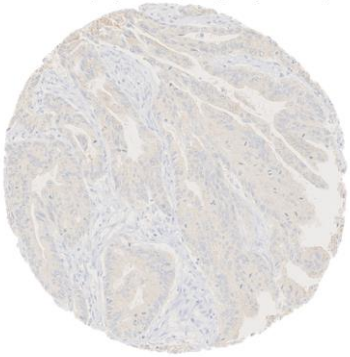
Lab ID	101	111	114	123	149	160	175	176	207	217	228	230	Mutation Status
1	U	U	N	U	U	U	U	N	U	U	U	U	WT
2	E	N	N	P	E	P	N	P	P	N	N	N	*V600E*
3	N	N	N	N	N	N	N	N	N	N	N	N	WT
4	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
5	P	P	P	P	P	P	P	P	P	E	P	P	*V600E*
6	N	N	N	N	N	N	N	N	N	N	N	N	WT
7	P	P	E	P	P	E	E	P	P	N	E	E	*V600E*
8	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
9	N	N	N	N	N	N	N	N	N	N	N	N	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	N	WT
12	N	U	N	N	U	N	U	N	N	N	N	U	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	N	WT
16	P	P	P	P	P	P	P	P	P	P	E	P	*V600E*
17	N	N	N	N	N	N	N	N	N	N	N	N	WT
18	U	U	U	U	U	U	N	U	U	N	U	U	*V600E*
19	N	N	N	E	N	N	N	E	N	N	N	N	WT
20	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	*V600E*
22	N	N	N	E	N	N	N	N	N	N	N	N	WT
23	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	WT
25	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
26	P	P	P	P	E	P	P	P	P	N	E	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	N	WT
29	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
30	P	P	P	N	P	P	P	P	P	E	E	P	*V600E*
31	N	U	U	N	U	N	N	E	N	N	N	U	WT
32	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
33	N	N	N	E	N	N	N	E	N	N	N	N	WT
34	P	P	P	P	P	P	P	P	P	P	E	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	N	WT
37	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*
38	N	N	N	E	N	N	N	N	N	N	N	N	WT
39	N	N	N	E	N	N	N	N	N	N	N	N	WT
40	P	P	P	P	P	P	P	P	P	P	P	P	*V600E*

Figure 1. Representative weak positive staining (Core 26) in select participants.

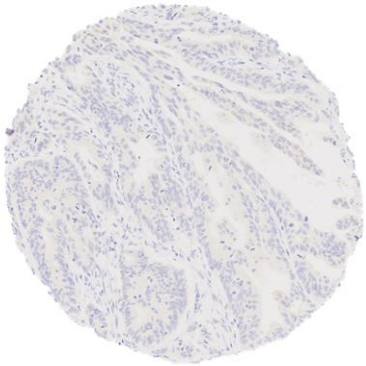
Lab 111 (Optimal)



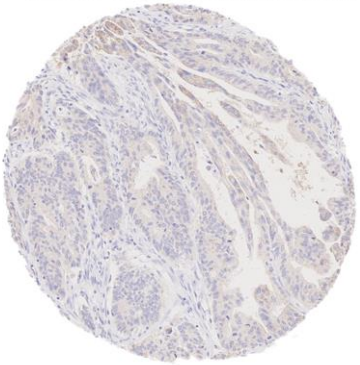
Lab 176 (Optimal; slightly weak)



Lab 228 (Adequate; weak)



Lab 230 (Optimal; slightly weak)



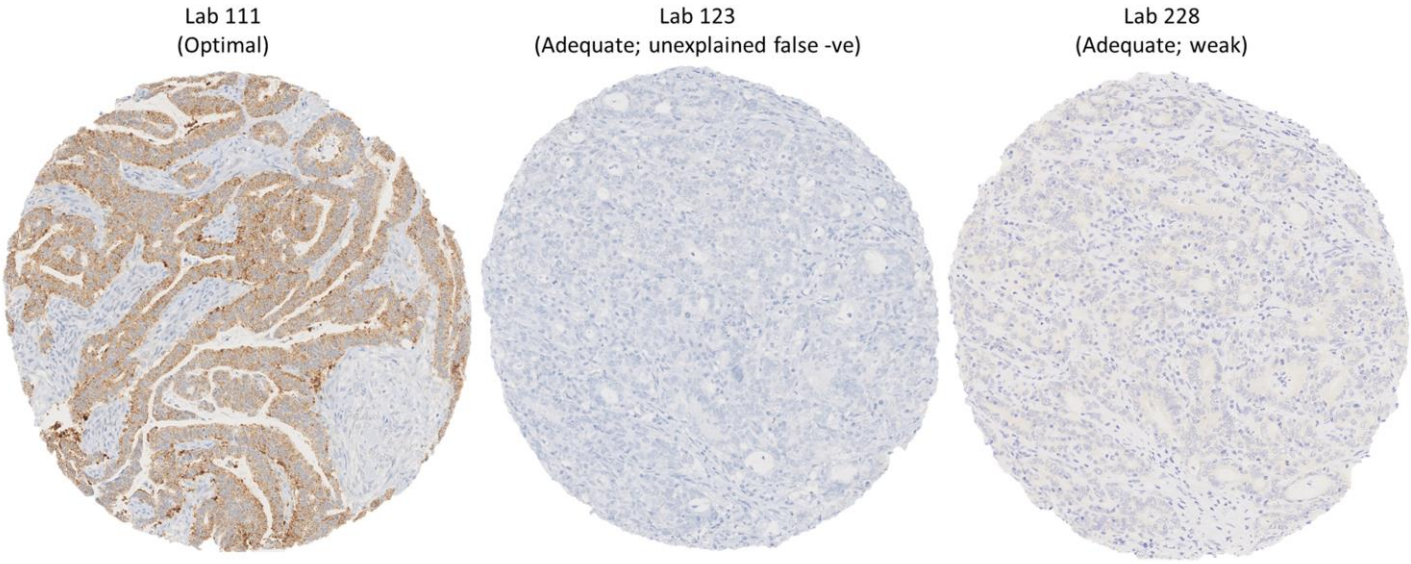


Figure 2. BRAFV600E staining in Core 30, a case with confirmed BRAFV600E mutation.

Supplementary Table 1 summarizes the reported staining protocols for BRAFV600E IHC, which can be referred to during validation or optimization of a staining protocol. Supplementary Table 2 summarizes descriptive statistics based on CPQA assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 3 provides the definitions of IHC Status and recommended participant action. Your regular participation in CPQA is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association canadienne des pathologistes.

This report has been updated with scanned images that were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.

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Table S1. Reported BRAFV600E IHC staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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)
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	1 Hr	V600E	1:200	ABCAM	GR323584020	15 MIN	DAKO Envision FLEX	N	N
111	Benchmark Ultra	commercial	Heat	48	VE1	predilute	Ventana	F30887	8	Optiview	Y	Y
114	Dako Omnis	LDT	Envision Flex TRS, High pH	60	VE1	1:200	Abcam	GR323584029	30	Envision FLEX DAKO Omnis	Y	N
123	Roche Benchmark Ultra	LDT	Roche CC1	64	V600E (VE1)	predilute	Roche	F27677	16	Roche OptiView DAB	Y	Y
149	Dako OMNIS	LDT	high pH OMNIS	20 min at 97 C	VE1	1:200	AbCam	GR32862733	20	EnVision Flex OMNIS	Yes	No
160	VENTANA	LDT	CC1	64MN	V600E	RTU	VENTANA	E17344	16 mn	optiview	Y	Y
175	Benchmark ULTRA	LDT	HIER	64	V600E	Pre-dilute	Roche	F27677	16	OptiDAB	Y	Y
176	Benchmark Ultra	commercial assay	CC1	64	VE1	1:200	abcam	GR3235840-3	32	Optiview	y	n
207	Omnis	LDT	high PH	15 minutes	V600E	1/400	abcam	GR3235840-23	15 minutes	magenta Flex detection System	Y	Y
217	ultra	ldt	hier ventana cc1	64	ve1	rtu	roche	f30887	36	optiview	y	y
228	Bond III	commercial assay	HIER	30 min	VE1	RTU	Ventana/ Roche	F09199	15 min	Bond polymer detection kit	N	N
230	Benchmark Ultra	LDT	HIER	32	VE1	predilute	Roche Diagnostics	G10183	16	Ultraview	Y	N

Table S2. Descriptive statistics based on CPQA assessment. Core 2 was excluded from analyses for reasons noted above.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	39	94.87	37	37/37 (100%)	1	1	1
111	39	89.74	35	35/35 (100%)	1	1	1
114	39	94.87	37	37/37 (100%)	1	1	1
123	39	94.87	37	36/37 (97%)	0.95	1	0.95
149	39	89.74	35	35/35 (100%)	1	1	1
160	39	94.87	37	37/37 (100%)	1	1	1
175	39	94.87	37	36/37 (97%)	0.95	1	0.95
176	39	97.44	38	38/38 (100%)	1	1	1
207	39	94.87	37	37/37 (100%)	1	1	1
217	39	97.44	38	35/38 (92%)	0.85	1	0.84
228	39	94.87	37	37/37 (100%)	1	1	1
230	39	89.74	35	35/35 (100%)	1	1	1

Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining).	**PASS**
Adequate	All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation.	**PASS**
Sub-optimal	None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation.	**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]**
Unsatisfactory	Technical issue (e.g. unsuitable antibody selection, etc.)	**N/A**

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

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