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canadian Immunohistochemistry Quality control

CIQC

Assessors' report for cIQc Run 79: IDH1 R132H

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Assessment performed on November 30, 2017, at Vancouver General Hospital

Recurrent mutations in the isocitrate dehydrogenase genes IDH1/2 have been found in infiltrative gliomas and are present in nearly all cases of secondary glioblastomas arising from low-grade gliomas, but rarely in the primary glioblastoma. IDH1/2 mutations are present in 60–80% of WHO grade II and III astrocytomas and oligodendrogliomas, and absent in non-neoplastic lesions, which can mimic tumours. Likewise, non-infiltrative gliomas, including pilocytic astrocytoma, dysembryoplastic neuroepithelial tumor, and ganglioglioma, do not contain IDH1/2 mutations. Missense mutation causing an arginine to histidine change in codon 132 (R132H) is most common and accounts for ~ 90% of IDH1 mutations in glioma. Immunohistochemistry for the mutant IDH1 R132H protein provides an essential adjunct in diagnostic neuropathology by increasing diagnostic confidence. This is particularly helpful in cases with presence of histologically-atypical cells of unknown etiology and limited availability of diagnostic tissue, such as brain biopsies, where spatial heterogeneity may result in a few neoplastic cells admixed with reactive, non-neoplastic cells.

■ Run 56 IDH1 Summary

Overview

Participating laboratories were asked to stain a tissue microarray consisting of 30 single-core gliomas that have been subjected to mutational analysis for IDH1 R132H by PCR. Slides 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 re-classified based on cIQc assessor consensus.

In general, a qualitative assessment of staining by all participating labs was performed and cIQc assessors primarily focused review on cores that were discordant with the PCR reference (R1). Specific comments from cIQc assessors are listed in the table below:

Lab ID	IHC Status	Comments
101	Optimal	
102	Adequate	Slight weak
103	Optimal	
109	Optimal	
110	Optimal	
111	Adequate	Slightly weak staining that is occasionally hazy; nice staining of control endothelial cells
112	Optimal	Very nice staining
114	Optimal	
120	Optimal	
123	Optimal	
126	Optimal	
136	--	
144	Optimal	

Lab ID	IHC Status	Comments
147	Optimal	
149	Optimal	
175	Sub-optimal	Weak staining that makes correct interpretation possible but more challenging in clearly positive cases; 2 false-negatives
191	Optimal	
194	Optimal	Equivocal cases only have 1 or 2 positive cells
202	Optimal	
207	Optimal	
217	Optimal	Very nice staining
222	Borderline Adequate	Unusual background staining in some cores leading to equivocal calls, false-positive staining in Core 13 neurons
228	Optimal	
234	Optimal	
236	Optimal	

*Based on cIQc assessment

Revised Garrattogram after cIQc *IDH1* R132H IHC assessment. Cores 1 and 8 were excluded from analyses due to loss of tumour in the core.

Lab/ Core	101	102	103	109	110	111	112	114	120	123	126	136	144	147	149	175	191	194	202	207	217	222	228	234	236	R1	
1	P	N	U	U	P	U	E	U	N	U	N	U	U	U	U	U	U	U	U	U	U	U	U	N	N	P	
2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	P	U	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	U	P	P	P	P	P	P	P	
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	
5	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	E	N	N	N	N	E	N	N	N	
6	P	E	P	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	
7	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
8	N	N	N	E	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	E	N	N	P	
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
11	P	U	U	P	P	P	P	P	P	P	P	P	U	P	P	N	P	P	P	P	P	P	P	P	U	P	
12	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	E	N	N	N	N	E	N	N	N	
13	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N	P	N	N	N	
14	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	E	P	P	P	P	P	P	P	P	P	P	
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	
18	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
19	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N	N	U	N	N	
20	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
22	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
23	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
24	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	
25	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
26	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
27	P	E	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
28	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	

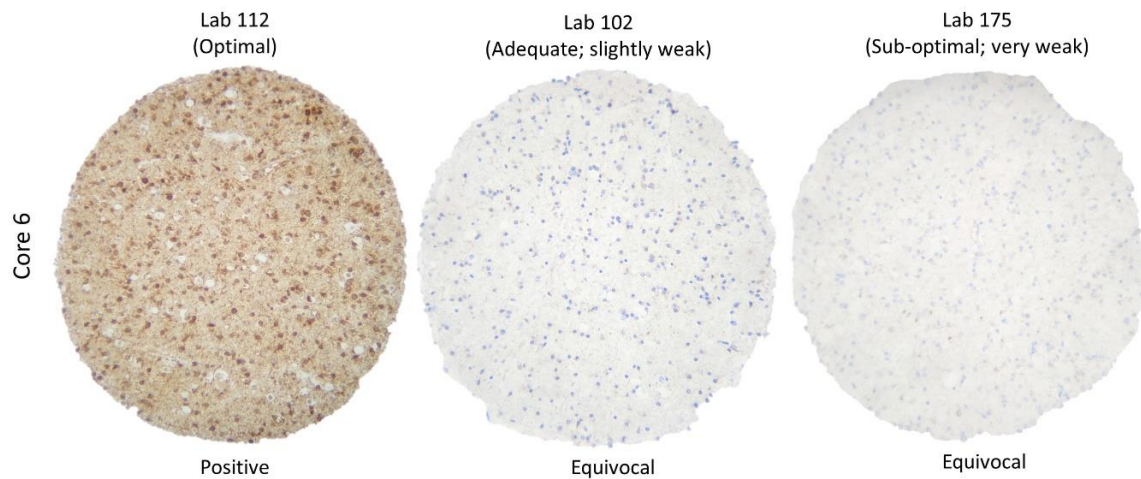


Figure 1. Representative images of the variable staining intensities observed across participants in an *IDH1* R132H-positive core (Core 6).

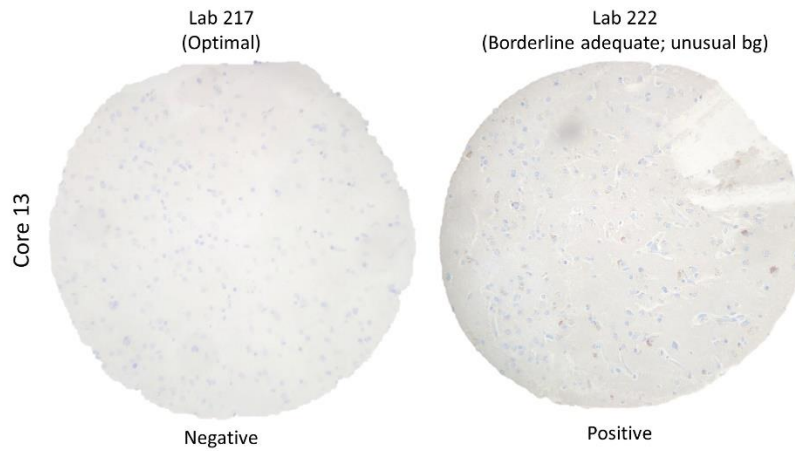


Figure 2. Representative images of the weak false positive staining observed in some cores of Lab 222 (bg = background).

Supplementary Table 1 summarizes reported staining protocols. 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 *IDH1* R132H IHC 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	CC1	32 min	H09	1:50	Dianova	161221/14	32min	OptiView	N	Y	DAB
102	DAKO PT - HIGH PH	20	H09	1:150	DIANOVA	1637/12	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64	H09 R132H	1/50	DIANOVA	1637/10	32	DAB	N	Y	COPPER
110	DAKO PT High ph 9.0@97 C	20 min	H09	1:200	Dianova	1637/06	30 min	Dako Envision Flex	N	N	DAB
111	HIER	48	H09	1/200	Histobiotech	15929/14	32	optiview	n	y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	15 minutes	H09	1:300	Dianova	151123/19	15 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	32	H09	1/50	Dianova	151123110	16	Optiview	N	Y	DAB
120	waterbath (TRS High)	20	H09	1:25	Dianova	16721/04	20	Envision Flex	Y	N	DAB
126	25" PT Module pH9	25	H09	1:500	Dianova	1637/12	30	Quanto	n	n	Beta Dab
136	Dako High	20	DIA-H09	1/25	DIANOVA	1733/08	10	ENVISION FLEX+	N	N	DAB
144	CC1	48 min.	H09	1:80	Dianova	15112	32 min.	Opti-View	No	Yes, copper	DAB
147	HIER PH 8	20	H09	1:125	DIANOVA	161221/15	15	POLYMER LEICA REFINE KIT	N	N	DAB
149	PT Link high pH	20 min at 97 C	DIA HO9 M	1:200	Dianova	1572210	30	EnVision Flex	Yes	No	DAB
175	HIER	48	HO0	1/50	DIANOVA	1265M/02	32	OPTI-DAB	Y	Y	DAB
191	CC1	64'	H09	1/50	Dianova	16829/13	32	optiview	N	N	DAB
194	HIER pH 9 (ER2)	20	H09	1/100	Dianova	1637/08	15	Leica Polymer Refine	N	N	DAB
202	ER 2	20	8O9	1/100	HISTOBIOTECH	1766-05	15	REFINE DETECTION SYSTEM	N	N	DAB
207	on-line CC1	40	H09	1/800	dianova	17123/03	60 minutes	Optiview	N	Y	DAB
217	HIER CC1	64	H09	1:100	Dianova	15316/01	32	Optiview	Y	Y	DAB
222	Ultra CC1	36	H09	1:5	Dianova	1766/4	28	Ultraview DAB	Y	Y	Copper
228	HIER in Bond Epitope Retrieval 2	30 min	H09	1:100	Dianova	151123/17	15 min	Bond Refine Detection System	N	N	DAB
234	PT Labvision	30	H09	40	Immunologic	161221/01	30	Powervision/poly-HRP-GAMs/RbIgG	no	Yes,CuSO4	DAB
236	HIER pH 9	20	H09	1:20	Dianova	1733/05	20	Agilent, EnVision+Dual Link	N	N	DAB