Assessors' report for cIQc Run 35: IDH1 R132H (January 2014)

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Assessment performed on Friday, May 2, 2014, at Vancouver General Hospital

Background

Recurrent mutations in the isocitrate dehydrogenase gene IDH1 have been found in infiltrative gliomas. They are present in nearly all cases of secondary glioblastomas arising from low-grade gliomas, but rarely in the primary glioblastoma. IDH1 mutations are present in 60–80% of WHO grade II and III gliomas, 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 mutations. In addition, presence of IDH1 mutation in a tumour correlates with improved prognosis. Mutation causing an arginine to histidine change in codon 132 (R132H) is most common and accounts for about 90% of IDH1 mutations in glioma. Immunohistochemistry for the mutant IDH1 R132H protein provides an essential adjunct in diagnostic neuropathology by increasing diagnostic confidence particularly in cases with presence of histologically-atypical cells of unknown etiology, 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.

Overview

Participating laboratories were asked to stain a tissue microarray consisting of 28 single-core gliomas that have been subjected to mutational analysis for IDH1 R132H by PCR. Overall, self-assessments from participating labs were excellent. Only the slide from Lab 191 was not returned to cIQc in time for the assessment meeting. Available slides from all other participating labs 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.

IDH1 R132H: Cores 2, 8, 10, 15, 17 and 22 were excluded from all analyses due to high dropout. Assessors noted that Core 3 possessed only a few positive tumour cells, which attributed to the variability observed in self-assessments by participants. 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).

Labs 102, 123, 126, 162 and 175 were deemed to have **optimal** staining by cIQc assessors. Due to generally weaker staining intensity overall Labs 103, 107, 114 and 125 were deemed to have **satisfactory** staining. Conversely, Labs 112 and 149 also had **satisfactory** staining due to slightly higher background staining compared to other labs. For instance, such background staining led to one false positive and a higher occurrence of equivocal results in Lab 112. Labs 101, 111 and 202 were considered to have **sub-optimal** staining compared to other labs. Whereas considerably weak staining intensity overall in Labs 101 and 111 led to false negatives in cores 11 and 25, Lab 202 had the opposite problem in which high background staining (typically granular) was observed in benign tissue elements and led to false positives in cores 12, 16 and 19. Protocol re-optimization is recommended for Lab 110 due to **unsatisfactory** staining that was observed to have led to significantly increased background staining and decreased overall sensitivity, leading to many discordant results.

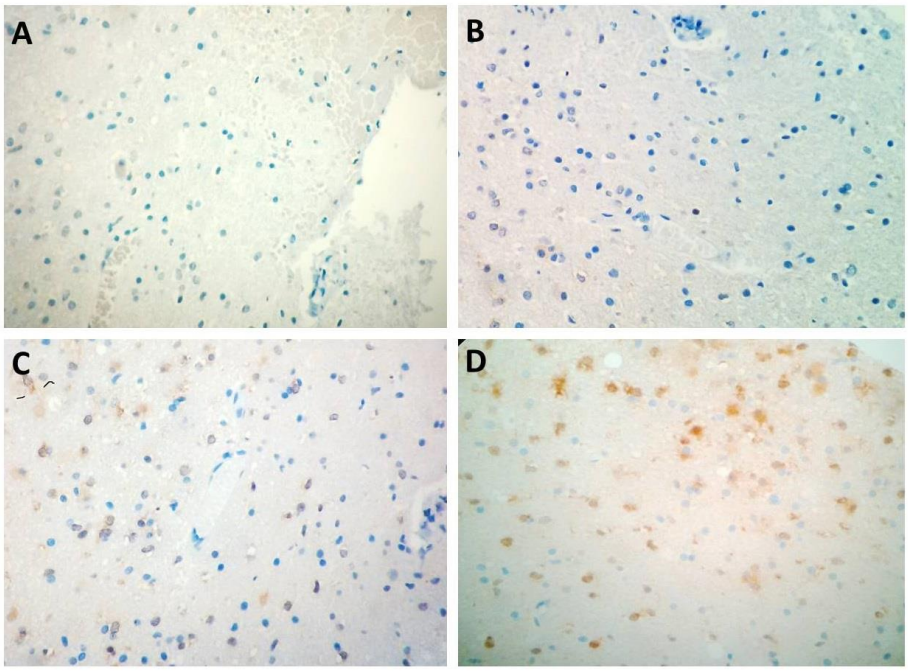

FIGURE 1. Variable IDH1 R132H staining of Core 25, a representative weakly positive core. (**A, B**) Overall weak staining intensity (Lab 101, **A**) or decreased sensitivity (Lab 110, **B**) led to a false negative result. (**C**) Observed optimal staining by Lab 123. (**D**) Generally high background staining observed in Lab 202.

The corrected Garrattogram for IDH1 R132H IHC results is provided in Supplementary Figure 1. Supplementary Table 1 summarizes kappa agreement values, sensitivity and specificity of each participating laboratory based on self-assessment and cIQc assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 2 summarizing staining protocols can also be found at the end of this document. 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.

Figure S1. Corrected Garrattogram after cIQc IDH1 R132H assessment.

Lab/Core	101	102	103	107	110	111	112	114	123	125	126	149	162	175	191	202	R1
1	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
~~2~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~
3	N	E	E	P	N	N	P	E	E	P	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
5	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P
6	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N
7	P	P	P	P	N	E	P	P	P	P	P	P	P	P	P	P	P
~~8~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~
9	N	N	N	N	U	N	E	N	N	U	N	N	N	N	N	N	N
~~10~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~
11	N	P	P	P	P	N	P	E	P	P	P	P	E	P	P	P	P
12	N	N	N	N	N	N	E	N	N	E	N	N	N	N	N	P	N
13	E	E	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P
14	P	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P
~~15~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~
16	N	N	N	E	E	N	N	N	N	N	N	N	N	U	E	P	N
~~17~~	~~U~~	~~N~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~	~~N~~	~~U~~	~~U~~	~~U~~	~~U~~	~~N~~	~~U~~	~~U~~	~~N~~
18	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P
19	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N
20	P	P	P	P	E	E	P	P	P	P	P	P	P	P	P	P	P
21	N	N	N	N	N	N	E	N	N	E	N	N	N	N	E	E	N
~~22~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~U~~	~~P~~
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
24	P	U	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
25	N	U	P	P	N	N	P	P	P	P	P	P	P	P	E	P	P
26	N	N	E	N	N	N	P	N	N	E	N	N	N	N	N	N	N
27	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
28	E	E	P	P	N	E	P	P	P	P	P	P	P	P	P	P	P

Table S1. IDH1 R132H descriptive statistics generated from self-assessments and cIQc assessment.

	Self-assessments										cIQc Assessment								
Lab ID	Total n	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa	Lab ID	Total n	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	22	100	22	19/22 (86%)	0.73	1	1	0.79	0.73	**101**	22	100	22	19/22 (86%)	0.73	1	1	0.79	0.73
102	22	90.91	20	20/20 (100%)	1	1	1	1	1	**102**	22	90.91	20	20/20 (100%)	1	1	1	1	1
103	22	100	22	22/22 (100%)	1	1	1	1	1	**103**	22	100	22	22/22 (100%)	1	1	1	1	1
107	22	100	22	22/22 (100%)	1	1	1	1	1	**107**	22	100	22	22/22 (100%)	1	1	1	1	1
110	22	95.45	21	14/21 (67%)	0.45	0.9	0.83	0.6	0.35	**110**	22	95.45	21	14/21 (67%)	0.45	0.9	0.83	0.6	0.35
111	22	100	22	19/22 (86%)	0.73	1	1	0.79	0.73	**111**	22	100	22	19/22 (86%)	0.73	1	1	0.79	0.73
112	22	100	22	21/22 (95%)	1	0.91	0.92	1	0.91	**112**	22	100	22	21/22 (95%)	1	0.91	0.92	1	0.91
114	22	100	22	21/22 (95%)	0.91	1	1	0.92	0.91	**114**	22	100	22	22/22 (100%)	1	1	1	1	1
123	22	100	22	22/22 (100%)	1	1	1	1	1	**123**	22	100	22	22/22 (100%)	1	1	1	1	1
125	22	95.45	21	21/21 (100%)	1	1	1	1	1	**125**	22	95.45	21	21/21 (100%)	1	1	1	1	1
126	22	100	22	22/22 (100%)	1	1	1	1	1	**126**	22	100	22	22/22 (100%)	1	1	1	1	1
149	22	100	22	22/22 (100%)	1	1	1	1	1	**149**	22	100	22	22/22 (100%)	1	1	1	1	1
162	22	100	22	20/22 (91%)	0.82	1	1	0.85	0.82	**162**	22	100	22	22/22 (100%)	1	1	1	1	1
175	22	95.45	21	21/21 (100%)	1	1	1	1	1	**175**	22	95.45	21	21/21 (100%)	1	1	1	1	1
191	22	100	22	22/22 (100%)	1	1	1	1	1	**191**	22	100	22	22/22 (100%)	1	1	1	1	1
202	22	100	22	19/22 (86%)	1	0.73	0.79	1	0.73	**202**	22	100	22	19/22 (86%)	1	0.73	0.79	1	0.73

Table S2. Reported IDH1 R132H staining protocols.

Lab ID	Clone	Dilution	Supplier	Ag Retrieval	Ab Incubation Time	Detection	Enhancement	Chromogen
101	H09	1:100	Dianova	CC1	32 min	Ventana Optiview	Copper	DAB
102	H09	1/250	Dianova	DAKO 3in1 High pH	30"	DAKO FLEX+	Copper Sulphate	DAKO DAB+
103	IDH R132H	1/50	DIANOVA	CC1 64,	32	ULTRA VIEW DAB	COPPER	DAB
107	H09	1:20	Dianova	Ventana CC1 32min	32min	Ventana Optiview DAB	none	DAB
110	H09	1:50	Dianova	High pH (pH 9)	30 min	Dako Flex	Mouse linker	DAB
111	H09	1/50	Histobiotec	CC1 - 36 minutes	32 minutes	Ultraview	----	Dab
112	H09	1:100	Dianova	CC1 30 min	40 min	iView	copper	DAB
114	H09	1/200	Dianova	CC1 32min	16min	Optiview-Ventana	Copper	DAB
123	H09	1:50	Cedarlane	CC1 standard	60 min	UltraView	none	DAB
125	111219/18	1/2000	dianova	ER1-30 (bond)	15 min	Bond Polymer Refine Detection	Bond DAB enhancer	DAB
126	H09	1:500 = 0.4ug/ml	OPTISTAIN	pH9.0 Tris-EDAT	30 minutes	Quanto polymer	None	Dako DAB+
149	DIA HO9 M	1:25	Dianova	PT Link, high pH	30 min	Envision Flex	Yes	DAB
162	H09	1:80	Dianova	Ventana CC1 48 min.	32 min, 36 C	Ventana OptiView	-	Ventana OptiView DAB
175	H09	1:200	Dianova	CC1	32 min	Optiview	None	DAB
191	H09	1/10	Dianova	CC1	32'	ultraview	none	DAB
202	H09	1/50	HISTOBIOTECH	HIGH PH	16 MIN	LEICA REFINE DETECTION KIT	NONE	DAB