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Assessors’ report for cIQc Run 44: *IDH1* R132H (April 2015)

Assessors: S Yip and J Won (recorder)

Assessment performed on Tuesday, June 2, 2015, 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.”

– cIQc Run 35 *IDH1*

**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. All participating labs returned slides to the cIQc office in time for the assessment meeting, and 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 following table:

Lab	IHC Status*	cIQc Comments
101	Optimal	
102	Optimal	
103	Optimal	
107	Optimal	
110	Optimal	



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111	Sub-optimal	Several false positives; unusual granular cytoplasmic staining; staining generally less crisp than others labs
112	Optimal	
114	Optimal	
123	Optimal	
125	Adequate	Staining generally less crisp than others labs
126	Optimal	
149	Adequate	Staining generally less crisp than others labs
162	Adequate	Staining generally weak compared to others labs
175	Optimal	
191	Adequate	Staining generally weak compared to others labs; unusual faint staining seen in endothelial cells
202	Adequate	Staining generally weak compared to others labs
215	Adequate	Nice staining of internal controls
217	Sub-optimal	Weak staining, leading to several false negatives

\*Based on cIQc assessment

The revised 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 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.



**Table S1. *IDH1* R132H descriptive statistics generated from 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
101	28	82.14	23	23/23 (100%)	1	1	1	1	1
102	28	82.14	23	23/23 (100%)	1	1	1	1	1
103	28	85.71	24	24/24 (100%)	1	1	1	1	1
107	28	85.71	24	24/24 (100%)	1	1	1	1	1
110	28	85.71	24	24/24 (100%)	1	1	1	1	1
111	28	82.14	23	20/23 (87%)	1	0.75	0.79	1	0.74
112	28	85.71	24	24/24 (100%)	1	1	1	1	1
114	28	85.71	24	24/24 (100%)	1	1	1	1	1
123	28	92.86	26	26/26 (100%)	1	1	1	1	1
125	28	96.43	27	27/27 (100%)	1	1	1	1	1
126	28	100	28	28/28 (100%)	1	1	1	1	1
149	28	96.43	27	26/27 (96%)	1	0.93	0.93	1	0.93
162	28	92.86	26	25/26 (96%)	1	0.92	0.93	1	0.92
175	28	96.43	27	27/27 (100%)	1	1	1	1	1
191	28	92.86	26	25/26 (96%)	1	0.93	0.92	1	0.92
202	28	92.86	26	26/26 (100%)	1	1	1	1	1
215	28	100	28	28/28 (100%)	1	1	1	1	1
217	28	96.43	27	24/27 (89%)	0.77	1	1	0.82	0.78

**Table S2. Reported *IDH1* R132H staining protocols.**

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	H09	1:50	dianova	14729/12	32 minutes	OptiView	N	Y	DAB
102	DAKO PT 3IN1 HIGH PH	10/20/20	H09	1/250	DIANOVA	1265/06	30"	DAKO FLEX	NO	YES CUSO4	DAB+
103	CC1	64 MINS	1449/13	1/50	DIANOVA	1449/13	32	ULTRAVIEW	N	Y COPPER	DAB
107	cc1 m Benchmark XT	32 min	H09	1:30	Dianova	14916/15	32 min	Optiview DAB	N	Y	DAB
110	PT - pH9	20	H09	1:200	Dianova	13101/02	30 min	Dako FLEX	No	No	DAB
111	CC1	48 min	H-09	1/50	Histobiotec	14916/04	32	Optiview	n	Copper	DAB
112	BOND ER 2 pH 9.0	15 minutes	H09	1:300	Dianova	141113/13	15 minutes	BOND POLYMER REFINE	NO	NO	DAB
114	CC!	32	H09	1:50	Dianova	1282123	16	OPITVIEW	N	Y	DAB
125	ER1-30 Leica Bond III	30 min	111219/18	1/1000	Dianova	14522/15	15 min	Bond Polymer Refine Detection	n	n	DAB
126	Pretreatment Module	25 min	H09	1:500	Dianova	13101/14	30 min	Thermo's Quanto Small Polymer	No	No	DAB
149	PT Link high pH 96 C	20 min	DIA H09 M	1:25	Dianova	14916/06	30	Envision Flex	Yes	No	DAB
162	Ventana CC1	48 min	H09	1:80	Dianova	R132H	32 min	Ventana OptiView DAB	-	-	DAB
175	Heat	48	H09	1/200	Dianova	1123	32	opti view	N	Y copper	DAB
191	CC1	30'	H09	1/10	dianova	14916/10	32'	ultraview	N	N	DAB
202	Bond ER2	20 min	H09	1/100	Histobiotec	13715102	15 min	Refine Detection kit leica	n	n	dab
215	CC1	64	H09	1/100	HistoBioTec	1123	32	Optiview	Y	N	DAB
217	Ventana CC1	36 min	H09	1:50	HISTONOVA	121211/14	32 min	Ventana ultraView	N	COPPER	DAB