

Assessors' report for cIQc Run 56: *IDH1* R132H (January 2016)

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**Background**

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.

**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. Most 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). Observed variable staining in Core 3 across participating labs was deemed to be due to poor sample fixation. **Optimal performance of nearly all participating labs during this challenge compared to previous *IDH1* challenges (Run 35 and 44) demonstrates the value of continued participation in proficiency testing.** Specific comments from cIQc assessors are listed in the following table:

Lab	IHC Status*	cIQc Comments
102	Optimal	Endothelial cells clearly negative, demonstrating good specificity. Staining is clearly cytoplasmic, making interpretation easy.
103	Optimal	
110	Optimal	High background noted in Core 3, but negative cores are very clean.
111	Optimal	Light counterstain, but nice internal controls observed.
112	Optimal	
113	Optimal	



**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
102	DAKO 3IN1 High pH	10/20/10	H09	1:200	DIANOVA	1265/06	30" RT	DAKO FLEX	NO	CUSO4	DAB+
103	CC1	64 MINS	H09	1/50	DIANOVA	15722/11	32	DAB	N	N	DAB
110	High ph 9.0	20 min	H09	1:200	Dianova	13101/02	30 min	DAKO ENVISION FLEX	N	N	DAB
111	CC1- HIER	48 min	H09	1/200	Dianova	14916/04	36 min	OPTIVIEW	N	Copper	DAB
112	Bond ER 2 pH 9.0	15 minutes	H09	1:300	DIANOVA	141113/13	15 minutes	BOND Polymer Refine Detection	no	no	DAB
113	High pH Target Retrieval Buffer	30'	R132H	1/80	Dianova	15529/13	30'	Flex	N	N	DAB
114	CC1	32	H09	1/50	Dianova	151123/10	16	Ventana Optiview	N	Copper	DAB
123	EDTA HIER	64	H09	1/50	Dianova	15929/13	60	Polymer	N	Y	DAB
125	Dako EnV FLEX High pH	30 min	H09	1/1000	Dianova	1452215	20 min	Dako EnV Flex	Y	N	DAB
126	PT Module, Tris/EDTA buffer, ph 9.0	40 minutes	H09	1:500	Dianova	1569/04	30 minutes	Quanto	Yes	No	DAB +
144	CC1	48 min.	DIA-H09	1:80	HistoBioTec	15112/14	32 min.	Optiview	No	Yes, Copper	DAB
149	PT Link high pH	20	DIA HO9 M	1:200	Dianova	01557/02	20	EnVison Flex	Yes	No	DAB
162	CC1 ventana	48 min	H09	1:80	Dianova	14916/17	32 min	Optiview Ventana	no	no	DAB
164	ultraCC1	64	H09	1:50	Dianova	1569-15	32	Optiview	N	N	DAB
175	HIER (CC1)	48	R132H	1:50	Dianova	1123	32	Ventana OptiView Kit	No	Yes (Copper)	DAB
191	CC1	32'	H09	1/25	Dianova	1569/07	32'	optiview	N	N	DAB
202	ER2 pH 9.5	20 min	h09	1/100	Dianova/histobiotech	1569-02	15 min	Leica Refine Detection kit	N	N	DAB
216	HIER	60	H09	1:20	Cedarlane/Dianova	1569/17	40	Ventana IView DAB Detection Kit	N	Y	DAB
217	HIER (CC1)	64	H09	1:100	Dianova	15316/01	32	Optiview	y	y	DAB