Assessors' report for cIQc Run 63: ATRX (June 2016)

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Assessment performed on Tuesday, October 25, 2016, at Vancouver General Hospital

Background

"The combined application of IDH1 R132H and ATRX immunohistochemistry and 1p/19q co-deletion analysis can significantly increase the diagnostic and prognostic accuracy of low grade gliomas. Constituting a key parameter in this integrated diagnosis, abrogated ATRX protein expression based on immunohistochemistry is used as a surrogate for ATRX mutation, which is strongly associated with IDH1/2 mutated astrocytomas and not oligodendrogliomas. ATRX immunohistochemistry can refine the diagnostic accuracy of low grade glioma; however, it is heavily influenced by the quality of tissue material, and interpretation is particularly challenging as nuclear positivity is seen in endothelial cells, entrapped neurons, microglia and reactive astrocytes."

- cIQc Run 49 ATRX Summary

Overview

Overall, self-assessments from participating labs were good. Independent review led to occasional 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. Core 3 has lost most cells with typical morphology, so it was excluded from all analyses. Cauterization of tissue in Core 12 led to degradation of ATRX antigenicity, so it was excluded from all analyses. Core 17 only had 1/3 of the core remaining on all slides, so it was excluded from all analyses.

Specific comments from cIQc assessors are listed in the following table:

Lab	IHC Status*	cIQc Comments
101	Optimal	
102	Optimal	Endothelial cells (positive internal control) stained weaker compared to other labs, which was likely the reason why the self-assessment had several equivocal scores that were considered positive after cIQc assessment.
103	Adequate	Very strong intensity staining, which has caused a lot of cytoplasmic background that was incorrectly interpreted as nuclear positivity in several cores during self-assessment.
110	Adequate	Weak staining compared to other labs, which resulted in several equivocal calls for cores that should have been clear positives (e.g. Core 23).
125	Optimal	
126	Optimal	Endothelial cell internal controls failed in Core 11, leading assessors to agree with the equivocal call during self-assessment.
149	Optimal	
175	Optimal	

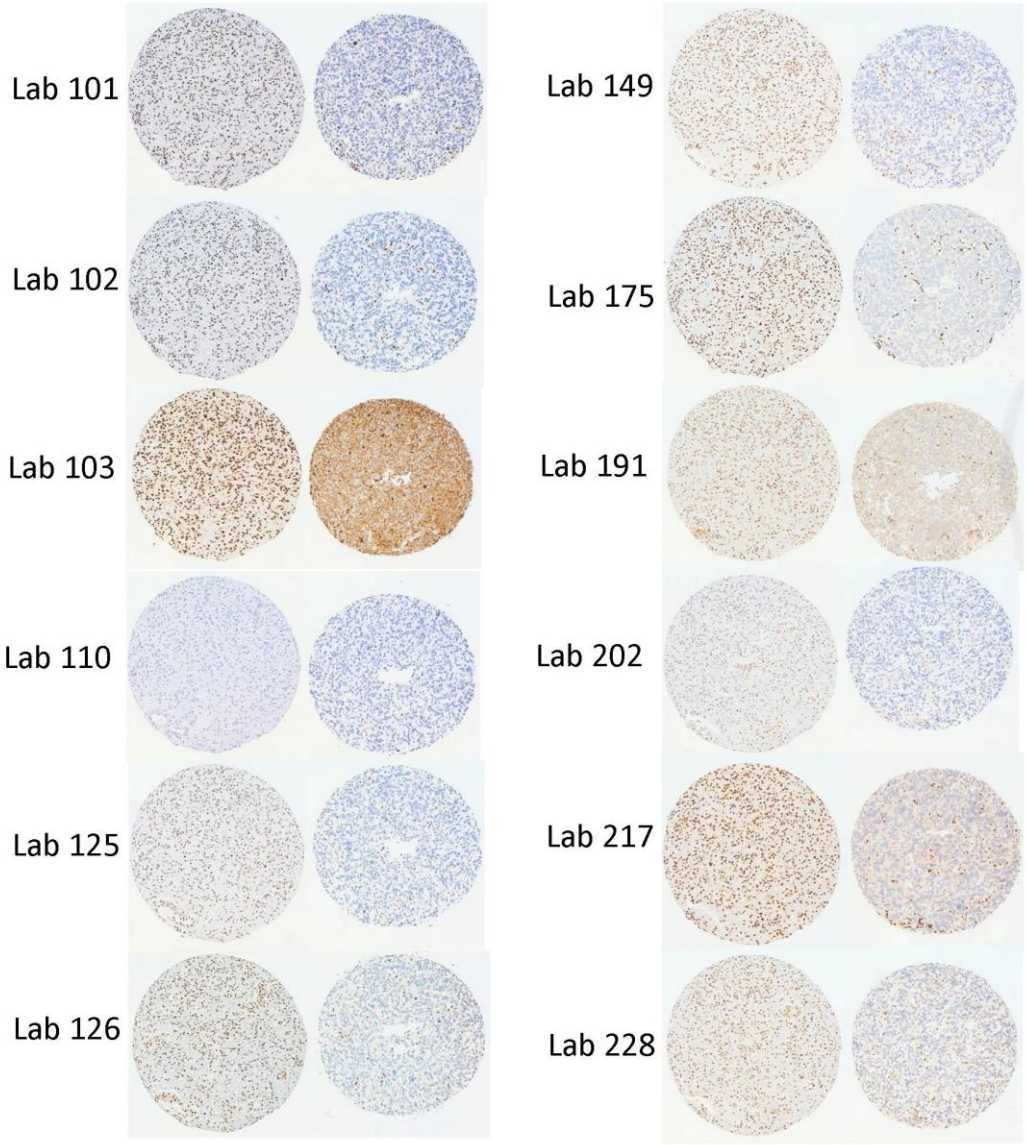
191	Optimal	Slight background staining
193	--	No slides available for review
202	Optimal	Slightly weaker staining intensity compared to other labs.
217	Optimal	Slight background staining
228	Optimal	

*Based on cIQc assessment

Conclusion

This second ATRX immunohistochemistry proficiency testing challenge has demonstrated that ATRX immunohistochemistry continues to be a challenge. The staining variability is illustrated below in Figures 1. Interpretation must be done by an experienced neuropathologist and continued participation in external quality assurance is necessary due to the additional level of interpretation required for ATRX immunohistochemical findings (e.g. the need to evaluate endothelial cell staining).

Figure 1. Variable IHC staining of ATRX in representative positive and negative cores.



The Garrattogram from cIQc-assessment is provided in Supplementary Figure 1. Supplementary Table 1 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. Garrattogram after cIQc-assessment of ATRX IHC. ***NOTE: Unsatisfactory results for ATRX IHC include cores with clear nuclear positivity in tumour cells BUT lack of staining in internal controls.***

Lab/ Core	101	102	103	110	125	126	149	175	191	193	202	217	228	REF	*IDH1* R132H Status	1p/19q Status
1	P	P	P	E	P	P	P	P	P	P	P	P	P	P	WT	
2	U	U	U	U	U	U	U	U	U	U	U	U	U	N	WT	Retained
3	E	P	P	N	N	P	E	P	E	U	N	P	E	P	R132H	Loss
4	U	U	U	U	U	U	U	U	U	U	U	U	U	E	WT	
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	R132H	Retained
6	P	P	P	P	E	P	P	P	P	P	P	P	P	P	WT	
7	N	E	E	N	N	N	E	N	N	U	E	N	N	E	R132H	
8	U	U	U	U	N	U	U	U	U	U	U	U	N	N	WT	
9	P	P	P	P	P	P	P	P	P	P	P	P	P	P		
10	U	U	U	U	U	U	U	U	U	U	U	U	U	U	WT	
11	N	U	N	N	U	U	N	N	N	U	N	N	N	N		
12	E	U	U	U	U	P	N	N	U	U	U	P	N	P	WT	
13	N	N	N	N	N	N	N	N	N	N	N	N	N	N	R132H	Retained
14	P	P	P	P	P	P	P	P	P	P	P	P	P	P	R132H	Loss
15	U	U	U	U	U	U	U	U	U	U	U	U	U	U	WT	
17	N	N	U	U	N	P	U	E	U	U	U	E	E	N	WT	
18	P	P	P	P	P	P	P	P	P	P	P	P	P	P	R132H	Loss
19	N	N	N	N	U	U	U	U	U	U	U	U	N	N		
20	U	U	U	U	U	U	U	U	U	U	U	U	U	P		
21	P	P	P	E	E	P	P	P	P	P	P	P	P	P	WT	
22	U	U	U	U	U	U	U	U	U	U	U	U	U	U	R132H	Retained
23	P	P	P	E	P	P	P	P	P	P	P	P	P	P	WT	
24	N	N	N	N	N	N	N	N	N	N	N	N	N	N	R132H	Retained
25	N	E	P	N	E	P	E	P	P	U	E	P	E	N		Retained
26	U	U	U	U	U	U	U	U	U	U	U	U	U	P	WT	
27	P	P	P	P	P	P	P	P	P	P	P	P	P	P	WT	
28	N	N	N	N	N	N	N	N	N	U	N	N	N	N	R132H	

Table S1. Reported ATRX IHC staining protocols.

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time (mins)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Ab Incubation Time	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	HEAT (CC1)	40	POLYCLONAL	1:200	SIGMA	J104367	32	OPTIVIEW	NO	COPPER	DAB
102	DAKO 3IN1 High pH	10/20/10	Polyclonal	1:400	Sigma	D96911	30" RT	DAKO FLEX	NO	CUSO4	DAB+
103	CC1	48 mins	Polyclonal	1/400	Sigma	E98955	1 hour	optiview	Yes	Copper	Dab
110	Dako PT, low pH	20 min @ 97C	rabbit polyclonal	1:200	Sigma Life Sciences	L104380	30 min at room temp	Dako Envision Flex	N	N	DAB
125	Dako EnV FLEX TRS high pH	30	polyclonal	1/300	Sigma	J104904	10 min	Dako EnV FLEX	n	n	DAB
126	microwave pressure cooker with citrate buffer, ph 6.01	35 minutes	rabbit/polclonal	1:450	Atlas-Sigma	J105268	30 minutes	MACH 4, HRP, Biocare	no	no	DAB +
149	PT Link high pH	20 min at 97 C	HPA001906	1:500	Sigma	10107434	20 min	EnVision Flex	Yes	No	DAB
175	HIER	48	ATRX	1/100	Sigma	J104904	60	Opti Kit	N	Y- Copper	DAB
191	CC1	60 min	Polyclonal	1/800	Sigma	A96586	60 min	Ultraview	Y	N	DAB
193	high pH	30 minutes at 97 deg	rabbit poly	1/400	sigma aldrich	D96911	20 min	Envision Flex plus	yes	no	DAB
202	leica ER1 ph 6.0	20 min	poly	1/400	sigma	j104367	15 min	Refine Detection kit Leica	no	no	DAB
217	HIER CC1	92	polyclonal rabbitt	1:200	sigma	E97092	120	Ultraview	Y	Y	DAB
228	HIER citrate buffer	20'	Rabbit polyclonal	1:100	Sigma	J105327	15'	Bond refined detection kit	N	N	DAB