



building towards

cIQc

canadian Immunohistochemistry Quality control

Assessors' report for cIQc Run 78: ATRX

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

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."

■ Run 63 Summary

Overview

More so than the previous ATRX challenge, variability of ATRX staining was evident. Once individually adjusting the threshold for considering positivity by reviewing each participants' overall staining across the entire tissue microarray, consistent assessment could be made from most slides submitted. Independent review led to occasional alteration of original self-reported results. Specific comments from cIQc assessors are listed in the following table:

Lab ID	IHC Status	Comments
101	Optimal	
102	Borderline Adequate	Moderate cytoplasmic background staining
103	Sub-optimal	Very high background leading to non-specific staining of cytoplasmic processes; false positives
110	Optimal	Slightly weak in some cores
112	Optimal	
114	Optimal	
120	Optimal	
125	Adequate	Slightly weak
126	Optimal	Slightly weak in some cores
147	Optimal	
149	Adequate	Slightly weak
175	Borderline Adequate	Moderate cytoplasmic background staining; Core 22 false positive
191	Adequate	Slight cytoplasmic background staining
193	Optimal	
194	Optimal	
202	Borderline Adequate	Weak intensity staining makes interpretation more challenging, yet slight cytoplasmic background
217	--	Slide not available for assessment
222	Adequate	Slightly weak
228	Adequate	Slight cytoplasmic background staining
234	Borderline Adequate	Unique membranous/cytoplasmic staining pattern seen in some cores that obscures nuclear interpretation; false negatives in top row may be attributable to technical artifact
236	Adequate	Slight cytoplasmic background staining

*Based on cIQc assessment

Figure 1. Representative images of variable staining observed across select participants in an ATRX-positive core (Core 5).

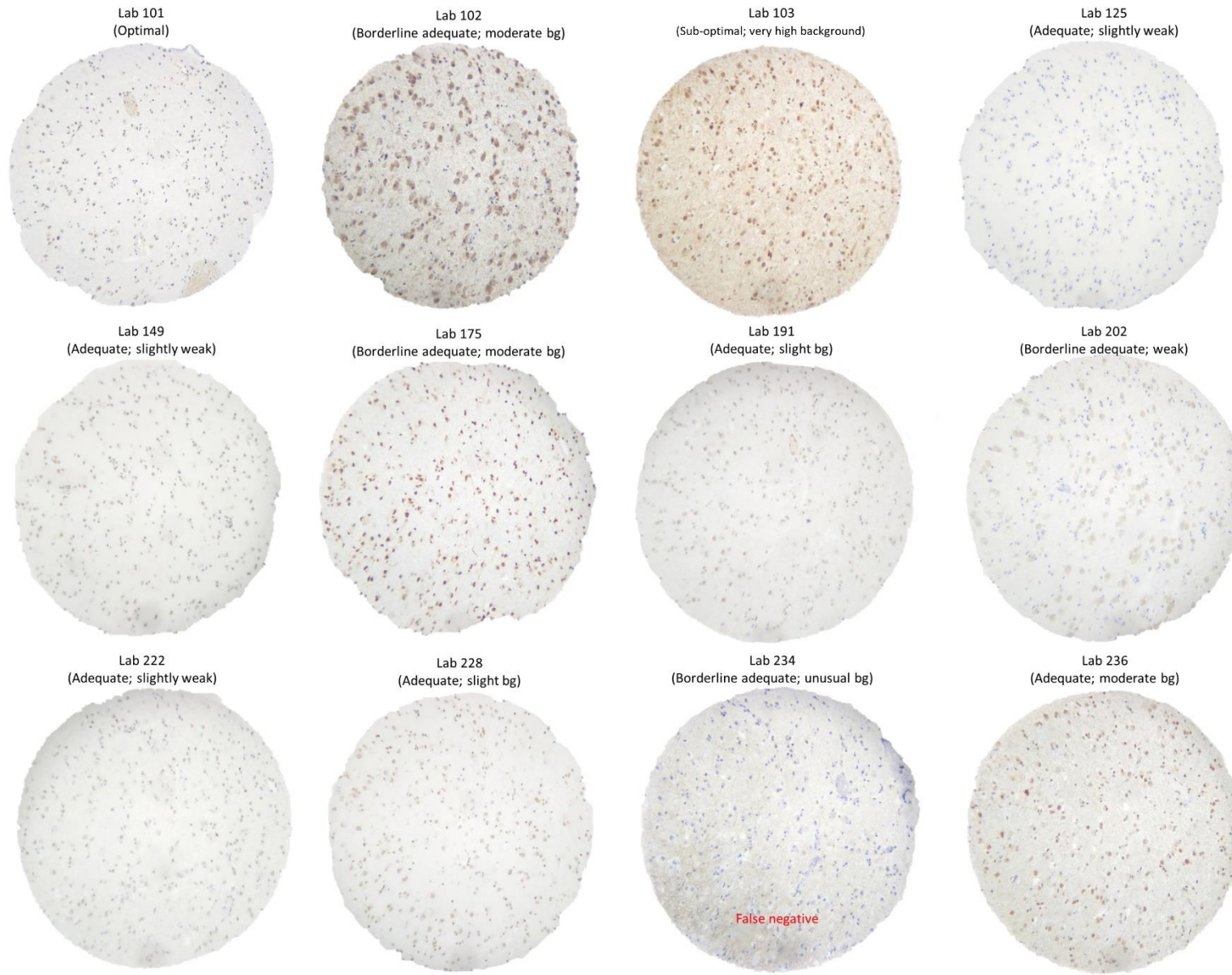
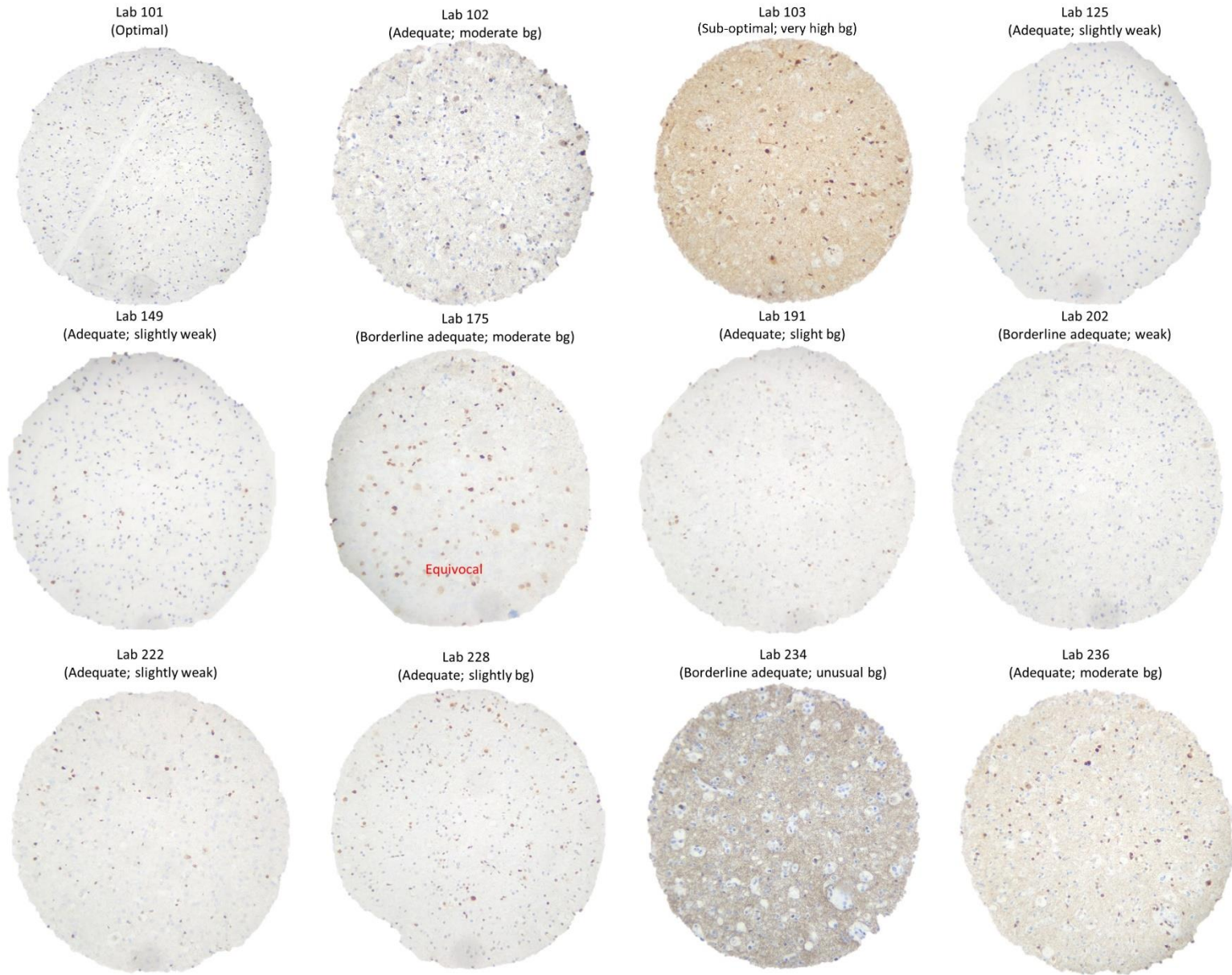


Figure 2. Representative images of variable staining observed across select participants in an ATRX-negative core (Core 6) (bg = background).



ATRX immunohistochemistry continues to present a challenge, particularly for interpretation which must be done by an experienced neuropathologist. Due to the additional level of interpretation required for ATRX immunohistochemical results (e.g. the need to truly evaluate staining in the different cells types within a sample), self-assessments from ATRX IHC proficiency testing can also be of value to participants. To emphasize a point mentioned previously, assessors needed to adjust the threshold for considering positivity by reviewing each participants' overall staining across the entire tissue microarray. This allowed for consistent assessment from most slides submitted despite considerable staining variability.

Supplementary Table 1 summarizing staining protocols can 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. Reported ATRX 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	40min	polyclonal	1:200	Sigma	BG106046	32min	OptiView	N	Y	DAB
102	DAKO PT - HIGH PH	20	POLYCLONAL	1:400	SIGMA	I105865	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	48	POLY	1/400	SIGMA	BB106933	60 MINS	OPTIVIEW	NO	COPPER	DAB
110	DAKO PT Low ph 6.0@97 C	20 min	rabbit polyclonal	1:200	Sigma	L104380	30 min	Dako Envision Flex	N	N	DAB
112	Bond epitope retrieval solution 1	60 minutes	polyclnal	1:250	Sigma Life Science	BG106188	30 minutes @97	Refine Detection System	none	none	DAB
114	CC1	32	Rabbit Polyclonal	1/200	Sigma	I105865	32	Optiview	N	Y	DAB
120	waterbath (TRS High)	20	polyclonal rabbit	1:500	Sigma/ Atlas Antibodies	J104696	20	Dako Envision Flex	N	N	DAB
126	Microwave Pressure Cooker with Citrate buffer, pH 6.00	35	RABBIT/POLYCLONAL	1:450	SIGMA	BD106732	30	MACH4 HRP, BIOCARE	N	N	DAB+
147	HIER ph 8	20	POLYCLONAL	1:250	SIGMA	N105341	15	Polymer Leica Refine Kit	N	N	DAB
149	PT Link high pH	20 min at 97 C	Poly R	1:500	Sigma HPA001906	BG106046	20	EnVision Flex	Yes	No	DAB
175	HIER	48	ATRAX	1/100	SIGMA	N105482	60	OPYI	Y	Y	DAB
191	CC1	64'	poly	1/800	sigma	a96586	60	ultraview DAB	Y	N	DAB
193	EDTA	30 Min.	Polyclonal	1/400	Sigma-Aldrich	L104260	20 Min.	20 Min.	Yes	N	DAB
194	HIER -ER2	15	Rabbit Polyclonal	1/100	Sigma	BG106046	15	Bond Polymer Refine DAB	N	N	DAB
202	ER1	20	HPA001906	1/400	SIGMA	105865	15	REFINE DETECTION SYSTEM	N	N	DAB
217	HIER CC1	92	polyclonal rabbit	1:200	Roche Ventana	J104951	120	Ultraview	Y	Y	DAB
222	Ultra CC1	36	Polyclonal	1:200	Sigma Aldrich	BF106523	16	Ultraview DAB	Y	Y	Copper
228	HIER in Bond Epitope Retrieval 1	20 min	Polyclonal	1:25	Sigma	BG106294	15 min	Bond Refine Detection System	N/A	N/A	DAB
234	PT Labvision / HIER M buffer	30	CL0537	40	AbCAM	GR 1310186-2 / 21-04-18	30	Powervision/poly-HRP-GAMs/RblgG	No	Yes, CuSO4	DAB
236	CC1	64	poly	1:400	Sigma	BB107076	56	UltraView	N	N	DAB