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**CIQC**

**canadian Immunohistochemistry Quality control**

Assessors' report for cIQc Run 75: p16 (May 2017)

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Assessment performed on September 27, 2017, at Vancouver General Hospital

## **Background**

Vulvar squamous cell carcinoma (VSCC) is associated with human papilloma virus (HPV) infection or unrelated to HPV, in which case there is often a history of lichen sclerosis. HPV-associated VSCCs tend to afflict younger women and have a characteristic “wart” or “basaloid” histological appearance, whereas HPV-independent VSCCs are more common in older women and have low-grade keratinizing morphology. Distinction between HPV-associated and HPV-independent VSCCs can be challenging based solely on morphological differences. p16 immunostaining, which detects protein overexpression in host cells infected by oncogenic HPV, can serve as a surrogate for determining HPV status. High levels of p16, a tumour suppressor, are triggered by deregulated cell proliferation resulting from the binding and inactivation of p53 and RB by the E6 and E7 proteins, respectively, of the oncogenic HPV virus. As such, p16 immunostaining has become an accepted surrogate for HPV molecular testing in cervical neoplasia, where essentially all squamous cell carcinomas are HPV-associated.

In cIQc Run 75 we assessed the performance of 51 laboratories in staining p16 for subclassification of VSCC into HPV-associated and HPV-independent cases. The ten cases used to build the tissue microarray in this challenge were selected from a larger cohort that has been previously described in Cheng *et al.* for which p16 expression and HPV status was extensively characterized.

Participating laboratories were asked to score p16 immunostaining according to the following scoring system:

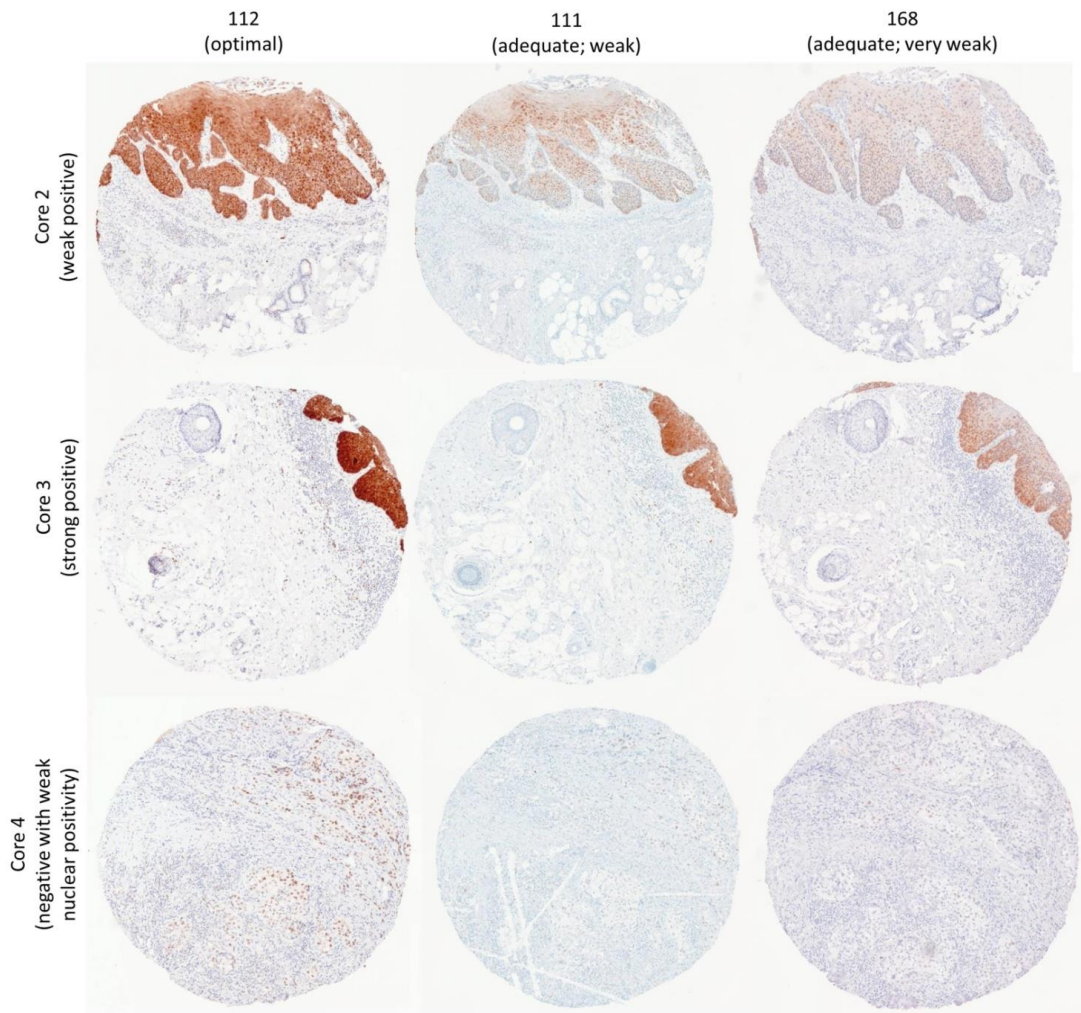
- **P** - Positive – Moderate to strong staining of the nucleus and cytoplasm of all cells in at least the lower-most third of the epithelium, as an indicator of oncogenic HPV infection (so-called “block” positivity)
- **N** - Negative – No staining or observed staining does not meet the pattern/threshold for positivity defined above
- **U** - core unsatisfactory for analysis (e.g. technical issues, no tumour or missing core)

## **Reference**

Cheng AS, Karnezis AN, Jordan S, Singh N, McAlpine JN, Gilks CB. p16 immunostaining allows for accurate subclassification of vulvar squamous cell carcinoma into HPV-associated and HPV-independent cases. *International Journal of Gynecological Pathology*. 2016 Jul 1;35(4):385-93.

At the time of assessment, all cores in the tissue microarray were reviewed. For cores where the assessment team altered the score from the self-assessment result, the new result was entered into the Garrattogram and lab performance calculated based on the results of cIQc assessment. Below are our subjective assessments of the performance of each laboratory where slides were available for review at the time of the assessment meeting. Participant-specific feedback is provided in the tables below:





**Figure 1.** Representative p16 staining. Immunostaining considered “Adequate” due to weaker intensity accounted for most results that were considered “adequate” rather than “optimal”. The weak nuclear staining in Core 4 was only infrequently observed; we have no explanation for this finding, but note that true positive staining should be both cytoplasmic and nuclear.

### Concluding comments

Immunostaining for p16 has emerged an important biomarker. The most common indication is in cervical biopsies where the differential diagnosis includes HSIL (CIN2/3). Such samples are not suitable for use in proficiency testing as they are too small, so we used a set of vulvar squamous cell carcinomas in this challenge, consisting of both HPV-associated and HPV-independent tumors. The overall results across Canada were very good, perfect concordance with the reference result, or at most one disagreement. The uniformity of the staining results was not surprising given that almost all labs use the same anti-p16 clone. The main issue identified in this run was that some labs had weak staining, such at correct identification of Core 2 as being HPV-associated VSCC was challenging. For labs with weaker staining they should be aware that the threshold for p16 positivity, as an indication of oncogenic HPV infection, is less than the very strong diffuse staining seen in many HPV-associated cervical and vulvar squamous cell carcinomas.

Supplementary Table 1 provides a summary of the staining protocols used in each participating laboratory. Supplementary Table 2 summarizing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen’s kappa for each participating laboratory can also be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Your regular participation in cIQc is greatly appreciated and we look forward to working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes in the future as we continue to improve our external quality assurance services.

**Table S1. Reported p16 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	32 min	E6H4	1:3 (RTU)	CINtec (VENTANA)	G07152	32min	Optiview	N	N	DAB
102	DAKO PT - HIGH PH	20	E6H4	1:2	VENTANA	G09130	30* RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	36	P16 (cINTEC)	PRE	VENTANA	G09513	12	DAB	N	Y	COPPER
104	HIER (Heat induced epitope retrieval)	30 min	E6H4	30 min	Roche	5.164301	30 min	PolymA <sup>re</sup>	yes	no	DAB
106	Performed on stainer	52 min	E6H4	n/a	Roche	G09130	20 min	Ultraview	no	copper sulphate	DAB
107	ultra cc1	76	E6H4	Pre-diluted	Ventana	G09130	32	Ultraview DAB	N	Y	DAB
109	HIER high pH (CC1)	16 MIN	E6H4	RTU	ROCHE	G08486	16 MIN	OPTIVIEW	N	Y	DAB
110	Dako PT high pH	20 min @ 97 C	E6H4	RTU	Roche	5.163901	30 min.	DAKO Envision Flex Flex 30	N	N	DAB
111	HIER	16	E6H4	PREDILUTE	VENTANA	G09130	8	OPTIVIEW	N	Y	DAB
112	Bond Epitope Retrieval Solution 2 pH 9.0	20 minutes	E6H4	1:1 of the RTU with DAKO antibody diluting buffer	Ventana Medical Systems (Roche)	5.164702	30 minutes	BOND Polymer Refine Detection	no	no	DAB
114	CC1	32	E6H4	1/2 (from RTU)	Ventana	G08486	16	Optiview	N	Y	DAB
115	Envision Flex Trs, High pH	30 min	Contec	1/5	Roche	F04670	20 mins	Envision Flex	N	N	DAB
116	CC1	36 MIN	E6H4	1/2	CINTEC ROCHE	170301	40 MIN	ULTRAVIEW DAB	N	Y	DAB
120	waterbath (TRS High)	20	E6H4	ready to use	Roche	5.164301	20	AutostainerLink48+ Flex+ 20 mouse	N	N	DAB
124	CC1	32 min	E6H4	PrA@-diluA@	Cintec	5.164702	20 min	Optiview	non	non	DAB
126	Tris/EDTA	40	E6H4	1:5	MTM Laboratories	5.162501	30	Dako Envision Plus	n	n	Dab Plus
127	HIER (AUTOMATED BENCHMARK ULTRA)	36 MIN	E6H4	PREDILUTE	VENTANA	G09130	32 MIN	ULTRAVIEW DAB	N	N	DAB
128	CC1	36 min	E6H4	Pre-dilute	Cell Marque	G08486	32 min	Ultraview	No	Yes	DAB
129	ER 2 -high pH retrieval	20	E6H4	1:3	Roche	5.164801	15	Bond Refine Detection Kit	N	N	DAB
132	High pH HEIR	20	p16 ink 4a	RTU	Cintec-Roche	5.170101	20	Envision Flex	N	N	DAB
133	HEIR	32	G175-405	1/50	BD Biosciences	5278514	32 minutes	polymer- Optiview	yes	no	dab
138	HIER High pH	20	INK4a	RTU	Cintec	5.170301	20	Dako Envision Flex	N	Y	DAB
141	HIGH PH	30 min	E6H4	RTU	Agilent	5.164301	30 min	polymer	no	no	DAB
144	CC1	32 min	E6H4	Pre-Dilute	Ventana	G08486	32 min.	Opti-View	No	Copper	DAB
145	HIER, CC1	40	E6H4A,φ	1/2	ventana	G04786	24	VENTANA XT OPTIVIEW	N	N	DAB
146	pH high	20	JC8	1:400	Santa Cruz Biotechnology	C3010	30	FLEX EnVision	n	n	DAB
147	HIER PH9	20	E6H4	1:3	ROCHE	5.162501	15	Polymer Leica Refine Kit	N	N	DAB
148	CC1	36 min	E6H4	RTU	Ventana	G09130	16 min	Ultraview	no	no	DAB
149	PT Link high pH	20 min at 97 C	E6H4	1:4	Ventana Roche CINtec	5.162501	20	EnVision Flex	Yes	No	DAB
150	cc1	60	E6H4	n/a	Roche Ventana	0	16min	UltraView	n	y	DAB
151	BUFFER PH 9.0	20 MIN	INK4A	1:3	CINTEC	5.162501	15 MIN	BOND DEFINE	N	N	DAB
155	CC1	60	E6H4	prA@-diluA@	Ventana	G00141	16	Ultraview dab	n	n	dab
157	CC1	32 MIN.	E6H4	PRE DILUTED	VENTANA	G 08486	16 MIN.	Benchmark XT OPTIVIEW	Y	Y	DAB
159	Flex TRS High	40 min.	E6H4	1/5 of RTU	Ventana	G09130	30 min.	Dako Flex	N	N	DAB
168	HIER	48	E6H4	1/4	Roche	Y01733	20	Envision Flex +	N	Y	DAB
175	HIER	16	p16	1 in 10	BD Pharmingen	6154652	32	Polymer	N	Y	DAB
183	Ultra CC1	64 minutes	(E6H4)	RTU	Ventana/Roche	G08486	20 minutes	Ultraview DAB	N	N	DAB
186	HIER	20	E6H4	1:4	CINtec Roche	5.162901	15	POLYMER	N	N	DAB
189	CC1	48	E6H4	pre-dilute	Ventana	F09797Z	12	OptiView	N	Y	DAB
194	HIER pH 9 (ER2)	20	JC8	1/1500	Santa Cruz	A2313	15	Leica Polymer Refine	N	N	DAB
199	HIER-Bond ER-2	20	INK4	1:50	BD Pharmingen	6154652	15	Bond Refine	n	n	DAB
202	HIER citrate pH 1	20	jc8	1/500	santa cruz	5.170101	15	Leica Refine detection kit	no	no	DAB
217	HIER	20	CINTEC	pre-dilute	Roche Ventana	610096	20	Ultraview	No	Yes	DAB
221	pH9 Tris/EDTA	25	E6H4	1:10	Ventana	G00141	30	Mouse EnVision	N	N	DAB
222	Ultra CC1	64	E6H4	1:2	Roche	5.170201	28	Ultraview DAB	N	Y	Copper
228	HIER inBond Epitope Retrieval 1	20 min	E6H4	Ready to Use	Ventana/ Cintec	5.164301	15 min	Bond refine Detection	N	N	DAB
230	HIER	40 MINS	CINTEC P16	NONE	VENTANA	Y01733	32 MINS	OPTIVIEW	N	N	DAB
231	ULTRA CC1	36	E6H4	PRE-DILUTE	ROCHE/VENTANA	G09130	36	ULTRAVIEW	N	N	DAB
234	PT Labvision/ HIER M buffer	30	MX007	60	Immunologic	1606216731/31-08-2018	30	Powervision?poly-HRP-GAMs/RblgG	no	Yes CuSO4	DAB
236	CC1	36	E6H4	RTU	Ventana/ROCHE	Y04586	32	UltraView	N	N	DAB

**Table S2. Descriptive statistics for p16 based on cIQC assessment. Cores 5 and 8 were excluded from analyses.**

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	8	100	8	8/8 (100%)	1	1	1	1	1
102	8	100	8	8/8 (100%)	1	1	1	1	1
103	8	100	8	8/8 (100%)	1	1	1	1	1
104	8	100	8	8/8 (100%)	1	1	1	1	1
106	8	100	8	8/8 (100%)	1	1	1	1	1
107	8	100	8	8/8 (100%)	1	1	1	1	1
109	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
110	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
111	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
112	8	100	8	8/8 (100%)	1	1	1	1	1
114	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
115	8	100	8	8/8 (100%)	1	1	1	1	1
116	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
120	8	100	8	8/8 (100%)	1	1	1	1	1
124	8	100	8	8/8 (100%)	1	1	1	1	1
125	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
126	8	100	8	8/8 (100%)	1	1	1	1	1
127	8	100	8	8/8 (100%)	1	1	1	1	1
128	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
129	8	100	8	8/8 (100%)	1	1	1	1	1
132	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
133	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
138	8	100	8	8/8 (100%)	1	1	1	1	1
141	8	100	8	8/8 (100%)	1	1	1	1	1
144	8	100	8	8/8 (100%)	1	1	1	1	1
145	8	100	8	8/8 (100%)	1	1	1	1	1
146	8	100	8	8/8 (100%)	1	1	1	1	1
147	8	100	8	8/8 (100%)	1	1	1	1	1
148	8	100	8	8/8 (100%)	1	1	1	1	1
149	8	100	8	8/8 (100%)	1	1	1	1	1
150	8	87.5	7	7/7 (100%)	1	1	1	1	1
151	8	100	8	8/8 (100%)	1	1	1	1	1
155	8	100	8	8/8 (100%)	1	1	1	1	1
157	8	100	8	8/8 (100%)	1	1	1	1	1
159	8	100	8	8/8 (100%)	1	1	1	1	1
168	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
175	8	100	8	8/8 (100%)	1	1	1	1	1
183	8	100	8	8/8 (100%)	1	1	1	1	1
186	8	100	8	8/8 (100%)	1	1	1	1	1
189	8	100	8	8/8 (100%)	1	1	1	1	1
194	8	100	8	8/8 (100%)	1	1	1	1	1
199	8	100	8	8/8 (100%)	1	1	1	1	1
202	8	100	8	8/8 (100%)	1	1	1	1	1
217	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71
221	8	100	8	8/8 (100%)	1	1	1	1	1
222	8	100	8	8/8 (100%)	1	1	1	1	1
228	8	100	8	8/8 (100%)	1	1	1	1	1
230	8	100	8	8/8 (100%)	1	1	1	1	1
231	8	100	8	8/8 (100%)	1	1	1	1	1
234	8	75	6	6/6 (100%)	1	1	1	1	1
236	8	100	8	7/8 (88%)	0.67	1	1	0.83	0.71