Summary Report – Run 121 p16

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Overview

p16 immunostaining can be used as a surrogate marker of high-risk oncogenic human papillomavirus (HPV) infection in genital squamous and glandular mucosa. For this challenge, cases and FISH results for HPV were kindly provided by BartsHealth in London, United Kingdom. The scoring system that was applied during assessment was as follows:

- **Abnormal (A)** - Abnormal (see below for definitions of abnormal)
- **Normal (N)** - Normal/Reactive or no tumour in the core
- **Unsatisfactory (U)** – insufficient tumour to evaluate, missing core, technical issues, etc.

Abnormal expression in squamous epithelium

- Abnormal expression is seen as confluent diffuse ("block") positive staining, characterised by the presence of ALL of the following:
  - Diffuse and strong nuclear and cytoplasmic expression
  - Involving basal and parabasal layers with upward extension
  - Upward extension must involve at least lower one-third of epithelial thickness
  - Abnormal expression must extend for at least 6 cells across

Abnormal expression in glandular epithelium

- Abnormal expression is continuous diffuse and strongly positive staining in glandular epithelial cells.

During CPQA assessment it was observed that the tumour for some cores had been cut through for several participants. The scoring system was altered to consider those cores as "normal" without tumour as oppose to "unsatisfactory", as participants were initially instructed.

Results

Core 1 showed relatively weak but true positive staining. Core 14 had only a small portion of tumour along the edge that was lost for many participants, leading to variable results. Core 17 was an HPV-independent invasive squamous cell carcinoma of the vulva that had many interpretive errors in self-assessment that were corrected during CPQA assessment. Core 21 was endocervical glandular epithelium showing patchy positivity, which is not the "block" positivity that correlates with high-risk HPV infection.

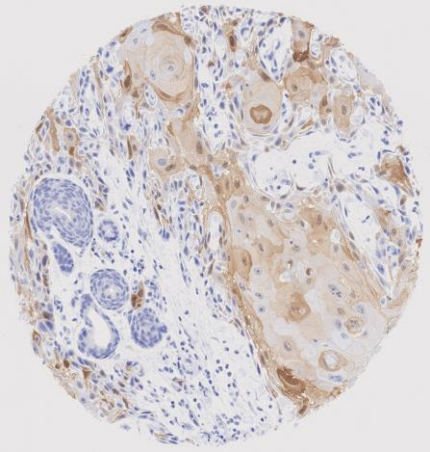

Figure 1. Representative image of p16 staining in an HPV-independent invasive squamous cell carcinoma of the vulva (usual clinical setting of elderly patient, with lichen sclerosis), which showed superficial but not basal p16 immunoreactivity, a pattern that does not correlate with high-risk HPV infection.

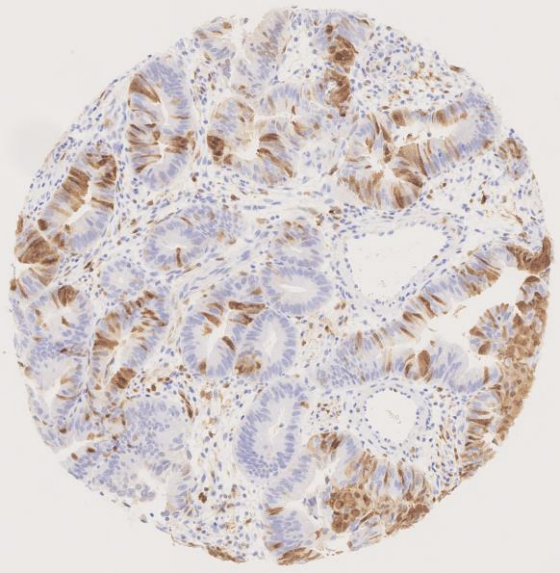

Figure 2. Representative image of p16 staining in endocervical glandular epithelium with patchy positivity, which is not the "block" positivity that correlates with high-risk HPV infection.

Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comment
101	Optimal	Slightly weak
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	Slightly weak
110	Optimal	
111	Optimal	
112	Optimal	Slight background
113	Optimal	Slightly weak
114	Optimal	Slightly weak
120	Optimal	
124	Optimal	
125	Adequate	Weak staining
126	--	Slide not available for assessment
127	Optimal	
128	Adequate	Weak staining
129	Optimal	
132	Optimal	
141	Optimal	
144	Adequate	Slightly weak; slight background
146	Optimal	

Lab ID	IHC Status*	Comment
147	Optimal	
149	Optimal	
151	Optimal	
159	Optimal	
160	Optimal	
175	Optimal	Slight background
180	Optimal	
183	Optimal	
186	Optimal	
190	Optimal	
194	Optimal	
198	Optimal	
199	Optimal	
202	Optimal	
207	Optimal	
209	Optimal	Slightly weak
220	Optimal	
228	Optimal	
230	Optimal	
231	Optimal	Slight background
234	Optimal	

*based on CPQA assessor consensus

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Garrattogram after CPQA assessment:

Lab/ Core	101	102	103	106	107	110	111	112	113	114	120	124	125	126	127	128	129	132	141	144	146	147	149	151	159	160	175	180	183	186	190	194	198	199	202	207	209	220	228	230	231	234	**Diagnosis**
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Cervical adenoca
2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	normal
3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	LSIL
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	normal
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	normal glands
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	normal
7	U	N	N	N	N	U	N	N	U	N	N	U	N	U	N	N	N	N	N	N	N	U	U	U	U	U	U	N	U	N	N	U	N	N	U	N	N	N	N	U	N	U	LSIL
8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	HSIL
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	normal
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	LSIL
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HSIL
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HSIL
13	A	A	A	A	A	A	A	A	A	A	A	A	A	N	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Cervical adenoca
14	N	N	A	N	A	N	A	N	N	A	N	A	N	U	N	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	adenoca in situ
15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	SCC CX
16	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	HSIL
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	VSCC (non-HPV)
18	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	sq metaplasia
19	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	endocx polyp
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	MGH
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	benign
22	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	reactive
23	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	benign
24	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	normal
25	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N	N	N	N	N	U	N	N	N	N	N	N	N	U	U	N	U	N	U	U	N	U	U	inflamed endocervix
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	?SMILE/HSIL
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	inflamed endocervix
28	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	normal/reactive
29	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Cervical adenoca
30	U	U	N	U	U	U	N	U	U	U	U	N	U	U	U	N	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	normal

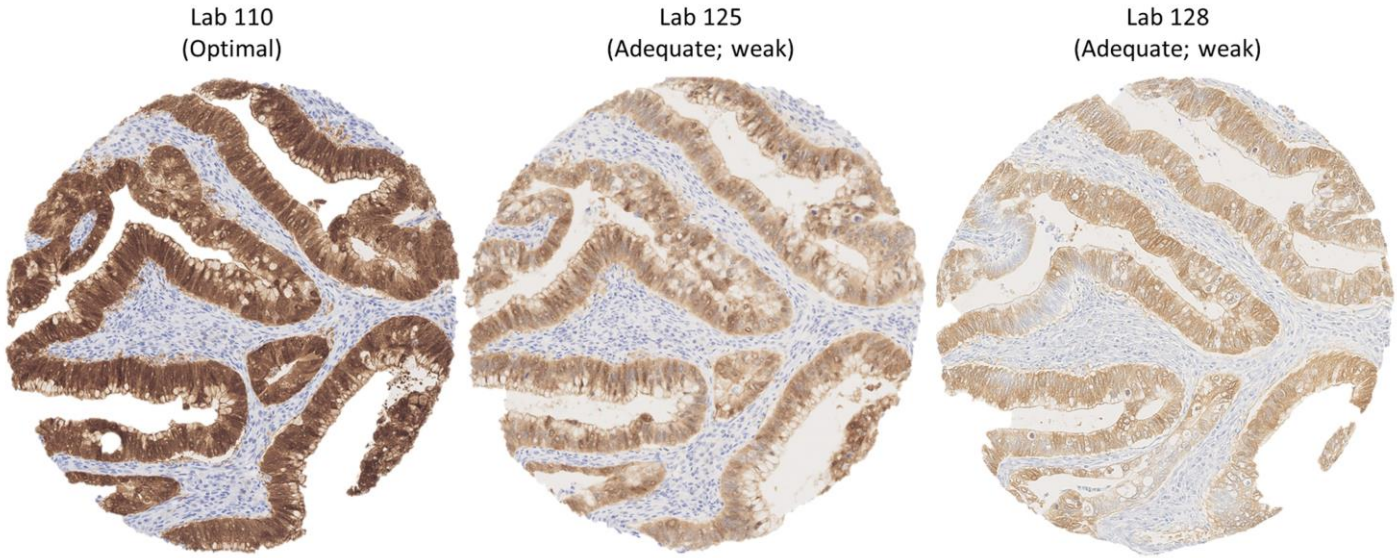

Figure 3. Representative images of the qualitative variability of p16 staining.

As with Run 102 p16, we refer you to detailed guidelines on interpretation of p16 immunohistochemistry in Anogenital Tract Neoplasia published by the British Association of Gynecological Pathologists and available to download online at https://www.thebagp.org/resources/. Supplementary Table 1 summarizes the reported staining protocols, which can be referred to during validation or optimization of a staining protocol. Supplementary Table 2 summarizes descriptive statistics based on CPQA assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 3 provides the definitions of IHC Status and recommended participant action. Your regular participation in CPQA is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association canadienne des pathologistes.

Scanned images in this report were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu

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Table S1. Reported p16 IHC staining protocols.

Lab ID	Platform/instrument	LDT or Kit	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)
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	E6H4	1:5	ROCHE DIAGNOSTICS	F11093	30 MIN	DAKO Envision FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retervial solution 9.0	20	E6H4	1:2	Roche	F30567	30" RT	Dako Envision Flex	N	yes CuSO4
103	Benchmark Ultra	Commercial	CC1	36	E6H4	Pre	ventana/roche	F20810	12	ultraview	no	y
106	Ventana Ultra	LDT	CC1 buffer	52	INK4a (E6H4)	RTU	Roche	G01371	20	Ultraview	No	Yes (CuSO4)
107	Dako Omnis	LDT	Dako FLEX TRS High pH	30	E6H4	1:6	Ventana	G01371	30	Dako FLEX	N	N
110	DAKO Autostainer Link 48	LDT	DAKO PT high pH 9.0 @ 97C	20 min	E6H4	ready to use	Roche	5.200501	30 min	DAKO ENVISION FLEX	N	N
111	Benchmark Ultra	commercial	HIER	32	E6H4	predilute	Ventana	F30567	32	Optiview	N	Y
112	BOND III	LDT	Bond Epitope Retrievial Solution 2 pH 9.0	20 minutes	6H12	RTU	Leica	67140	15 minutes	BOND Polymer Refine Detection	none	none
113	Dako OMNIS	LDT	High pH	30 min	E6H4	1/5	Roche	G01371	27.5 min	Dako Envision Flex HRP	N	N
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	E6H4	1:5	Ventana Roche	F30567	30	Envision FLEX DAKO Omnis	Y	N
120	Autostainer Link48	LDT	HIER Waterbath	20	E6H4	1/6	ROCHE	5.200501	30	Dako Envision Flex	Y	N
124	Benchmark Ultra	LDT	CC1	32	E6H4	PrÃ©-diluÃ©	Cintec par Roche	5.200501	20	Optiview	n	n
125	Dako Omnis	LDT	HIER	30	E6A4	1/24 of RTU	Roche	5200501	30	EnVision Flex	Y	N
126	Biocare intelliPATH	LDT	Tris/EDTA	20'	E6H4	1:2	Roche (Ventana)	F00314	30'	Envision+	N	N
127	VENTANA BENCHMARK	LDT	HIER	36 MIN	E6H4	PREDILUTE	VENTANA	F30598Z	32 MIN	ULTRAVIEW DAB	N	Y
128	Ventanna/BenchMark Ultra	LDT	Utra CC1	36 min	E6H4	RTU	Cell Marque	G01371	32	Ultra View Universal DAB Detection Kit	No	Yes
129	BOND III	LDT	ER 2- high pH retrieval	20	INK4a	1:3	ROCHE	5.200501	15	bond refine detection kit	N	N
132	Dako Autostainer	Commercial assay	High pH	20	INK4a	RTU	Roche/Cintec	5.200501	20	Envision flex	N	N
141	Autostainer Link 48	LDT	HIER	30 min	E6H4	RTU	Agilent/Dako	5.200501	30 min	Polymer	N	N
144	Dako Omnis	Commercial Assay	HIER	40 min	E6H4	1:5	Roche	121473	10	Flex	y	n
146	Autostainer Link 48	N/A	HIER	20	INK4a	1/6	CINtec	5.200501	30	EnVision FLEX	y	n
147	LEICA BOND 3	LDT	ER PH 8	20	E6H4	1:3	CINTEC	200501	15	LEICA REFINE KIT	N	N
149	Dako OMNIS	LDT	high pH OMNIS	20 min at 97 C	p16INK4	1:4	Roche	519200	26	EnVision Flex OMNIS	Yes	No
151	BOND 111	COMMERCIAL	HIER 2	20 MIN	INK4A	1:2	CINTEC	5.200501	15 MIN	BOND REFINE	N	N
159	Autostainer 48 Link	Commercial	Flex TRS High	40 min.	E6H4	1/6	Ventana	00F30567	30	Dako Flex	N	N
160	VENTANA	LDT	CC1	36mn	E6H4	RTU	VENTANA	5.200501	32MN	ultra-view	N	Y
175	Benchmark Ultra	Commercial	HIER	48	E6H4	pre-dilute	Roche	90137I	12 min	OPTI-DAB	N	Y (copper)
180	Ventana Ultra	LDT	CC1	64 min	E6H4	RTU	Ventana	F30567	32 min	Optiview	N	Y
183	Ventana Benchmark	LDT	ULTRA CC1	64	E6H4	RTU	VENTANA	G01371	20	ULTRAVIEW	N	N
186	L	LDT	HIER	20	16P04,JC2	NEAT	CANCER DIAGNOSTICS	5829BKD15	15	BOND POLYMER REFINE DETECTION	N	N
190	Ventena Ultra	LDT	HIER	36	E6H4	RTU	Roche	F11089	32	iView DAB	N	N
194	LEICA BOND III	LDT	HIER ER2 (high pH)	20	JC8	1/1500	Santa Cruz	A2313	15	Refine DAB	N	N
198	Dako-Omnis	LDT	HIER	30	CINtec	1/2	Ventana	F30567	30	Agilent Envision Flex	y	n
199	Bond-III	Commercial	Heat (ER-2)	30	E6H4	1:12	Roche	E31896	15	Bond Refine-DAB	n	n
202	DAKO Autostainer	LDT	HIER PH 6.0	10	E6H4	RTU	Cintec	5.200501	15	Bond refine detection kit	N	N
207	Benchmark ultra	ldt	CC1	CC1-24	CINtec	prediluted	Ventana	G01371	8 MINUTES	Optiview	N	Y
209	Dako Autostainer 48	commercial	HIER	20	805-4713	1:2	Roche Diagnostic	G01371	20	Flex polymer	N	N
220	Ventana BenchMark Ultra	commercial assay	HIER	36	E6H4	PRE DILUTE	VENTANA	G01371	32	Ventana ultraview	N	Y
228	Bond III	commercial assay	HIER using Bond Epitope Retrieval 2	20 min	16PO4,JC2	RTU	Bio SB/Cancer Diagnostics	58290JB19	15 min	Bond polymer detection kit	N	N
230	Benchmark Ultra	LDT	HIER	40	E6H4	predilute	Roche Diagnostics	F30567	32	OPTIVIEW	N	N
231	Ventana BenchMark Ultra	LDT	HIER	36 mins	E6H4	RTU	ROCHE/VENTANA	F30567	36 MINS	ULTRAVIEW (VENTANA)	N	Y
234	OMNIS	LTD	HIER / HIGH	30	MX007	2000	IMMUNOLOGIC	80119	20	ENVISION	Y	N

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Table S2. Descriptive statistics based on CPQA assessment. As the tumour in Core 14 was cut through for a handful of participants, the result in this core for these participants was categorized as "unsatisfactory" instead of "normal" for the purpose of this analysis.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	30	80	24	24/24 (100%)	1	1	1
102	30	83.33	25	25/25 (100%)	1	1	1
103	30	90	27	27/27 (100%)	1	1	1
106	30	83.33	25	25/25 (100%)	1	1	1
107	30	86.67	26	26/26 (100%)	1	1	1
110	30	80	24	24/24 (100%)	1	1	1
111	30	90	27	27/27 (100%)	1	1	1
112	30	83.33	25	25/25 (100%)	1	1	1
113	30	80	24	24/24 (100%)	1	1	1
114	30	86.67	26	26/26 (100%)	1	1	1
120	30	83.33	25	25/25 (100%)	1	1	1
124	30	86.67	26	26/26 (100%)	1	1	1
125	30	83.33	25	25/25 (100%)	1	1	1
126	30	76.67	23	22/23 (96%)	0.86	1	0.89
127	30	83.33	25	25/25 (100%)	1	1	1
128	30	90	27	27/27 (100%)	1	1	1
129	30	90	27	27/27 (100%)	1	1	1
132	30	90	27	27/27 (100%)	1	1	1
141	30	83.33	25	25/25 (100%)	1	1	1
144	30	93.33	28	28/28 (100%)	1	1	1
146	30	93.33	28	28/28 (100%)	1	1	1
147	30	90	27	27/27 (100%)	1	1	1
149	30	90	27	27/27 (100%)	1	1	1
151	30	90	27	27/27 (100%)	1	1	1
159	30	86.67	26	26/26 (100%)	1	1	1
160	30	90	27	27/27 (100%)	1	1	1
175	30	90	27	27/27 (100%)	1	1	1
180	30	93.33	28	28/28 (100%)	1	1	1
183	30	90	27	27/27 (100%)	1	1	1
186	30	93.33	28	28/28 (100%)	1	1	1
190	30	93.33	28	28/28 (100%)	1	1	1
194	30	90	27	27/27 (100%)	1	1	1
198	30	90	27	27/27 (100%)	1	1	1
199	30	90	27	27/27 (100%)	1	1	1
202	30	90	27	27/27 (100%)	1	1	1
207	30	90	27	27/27 (100%)	1	1	1
209	30	93.33	28	28/28 (100%)	1	1	1
220	30	90	27	27/27 (100%)	1	1	1
228	30	90	27	27/27 (100%)	1	1	1
230	30	86.67	26	26/26 (100%)	1	1	1
231	30	93.33	28	28/28 (100%)	1	1	1
234	30	86.67	26	26/26 (100%)	1	1	1

Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining).	**PASS**
Adequate	All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation.	**PASS**
Sub-optimal	None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation.	**PASS, Conditionally[1]**
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	**FAIL[2]**
Unsatisfactory	Technical issue (e.g. unsuitable antibody selection, etc.)	**N/A**

1 – A one-time sub-optimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".

1,2 – Please contact the CPQA for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.