Canadian Pathology Quality Assurance
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Assessors' report for Run 108: p53

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Assessment performed on May 16, 2020, at Vancouver General Hospital, British Columbia, Canada

Overview

TP53 mutational status and its surrogate, p53 immunostaining, in ovarian or endometrial carcinoma is a marker of histotype (e.g. low-grade endometrioid carcinoma of the endometrium vs. serous carcinoma of the endometrium, low-grade serous carcinoma of the ovary vs. high-grade serous carcinoma of the ovary), and for endometrial carcinoma is an independent marker of molecular subtype (p53abn, corresponding to The Cancer Genome Atlas Copy number-high subtype). Interpretation of p53 immunostaining is more complex than most markers, with either complete absence of staining or strong nuclear staining in at least 80% of cells providing evidence of p53 mutation (so called **"all or nothing" staining** pattern). Moderate to strong cytoplasmic staining without nuclear staining can also correlate with underlying mutations in *TP53*, but is very uncommon. p53 wildtype staining can be of variable intensity with weak to moderately intense staining in 1-80% of cells. In general, the higher the proliferation index, the greater the p53 staining in tumours/tissues with wildtype *TP53* (for example, basal keratinocytes of normal skin show variable p53 positivity while the mitotically inactive superficial keratinocytes are negative).

Results

Overall, the technical quality of staining for p53 was excellent for participating labs. The tissue microarray used for assessment consisted of ovarian carcinomas where *TP53* mutation status was known for most tumours (courtesy of Dr. Martin Kobel, University of Calgary). p53 immunostaining should be a specific (approaching 100%) but not completely sensitive (approximately 95%) marker of underlying mutation in *TP53*.

Several cores in the tissue microarray had notable issues: core 1 showed over-expression but of relatively weak intensity and large necrotic areas; core 8 had few tumour cells; core 23 had many intratumoral lymphocytes that were staining positively, in a tumour where there was loss of expression in the tumour cells, resulting in some labs interpreting it as wildtype staining; core 37 showed weak staining (wildtype or equivocal for overexpression) and is an example of a tumor with a *TP53* mutation where p53 immunostaining does not clearly reflect the underlying mutation, something that we see in approximately 5% of ovarian or endometrial carcinomas with a *TP53* mutation.

Participant-specific feedback for p53 IHC is summarized below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Optimal	Slightly weak; as well, some cores e.g. core 6, show faint staining of tumour cell nuclei, of weaker intensity than staining of internal control cells (see photomicrographs below). This should be interpreted as abnormal/absent p53 staining
103	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
110	Adequate	Strong counterstain
112	Optimal	
113	Optimal	
114	Optimal	
120	Optimal	
125	Optimal	
127	Optimal	
128	Adequate	Weak staining
132	Adequate	Weak staining; strong counterstain
141	Adequate	Weak staining
144	Adequate	Weak staining
147	Optimal	
148	Adequate	Weak staining
149	Optimal	
151	Optimal	

Lab ID	IHC Status*	Comment
159	Optimal	
160	Optimal	
168	Adequate	Weak staining
175	Optimal	
176	Optimal	
183	Adequate	Weak staining
186	Optimal	
192	Adequate	Weak staining
194	Optimal	
198	Optimal	
202	Optimal	
207	Optimal	
209	Adequate	Weak staining
217	--	Slide not available for assessment
220	Adequate	Weak staining
228	Adequate	Weak staining
230	Optimal	
231	Optimal	
234	Adequate	Weak staining; strong counterstain
236	Optimal	

*based on assessor consensus

Garrattogram after CPQA assessment of p53 IHC:

Lab/ Core	101	102	103	106	107	109	110	112	113	114	120	125	127	128	132	141	144	147	148	149	151	159	160	168	175	176	183	186	192	194	198	202	207	209	217	220	228	230	231	234	236	R1	TP53 mutation status	Histotype
1	N	A	N	N	N	U	N	N	A	N	A	N	A	A	N	N	N	A	N	A	N	N	A	N	U	N	N	A	N	N	N	N	A	N	A	A	A	N	A	N	A	A	Missense	EC
2	F	A	A	A	A	U	U	F	A	A	A	A	A	F	F	A	A	A	A	A	A	A	F	A	F	A	U	F	A	A	F	A	A	A	F	U	F	A	A	A	A	NA	MC
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	U	N	U	N	U	N	U	N	N	U	U	N	N	N	N	F	N	U	F	N	N	N	N	Wild type	EC
4	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	N	N	N	N	N	N	N	NA	CCC
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	NA	CCC
6	A	A	A	A	A	A	A	A	A	A	A	A	A	F	F	F	A	A	A	A	F	A	F	A	A	A	A	A	A	A	A	A	F	N	A	F	A	A	A	A	A	F	Splice site	HGSC
7	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	N	N	N	N	N	Wild type	EC
8	U	U	N	N	U	U	U	N	A	U	U	U	U	F	U	A	F	U	N	U	U	N	A	F	U	N	A	N	A	U	U	U	U	A	U	F	N	U	U	F	U	A	Stopgain	HGSC
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	NA	CCC
10	N	N	F	F	N	U	F	N	N	N	F	N	F	F	N	F	F	N	F	F	N	N	N	N	F	N	F	U	F	N	F	N	F	N	N	F	U	F	N	F	N	N	NA	CCC
11	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	NA	HGSC
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	F	N	N	N	N	N	N	N	N	N	N	N	N	N	NA	LGSC
13	A	A	A	A	A	A	A	A	A	A	A	A	A	F	F	A	A	A	F	A	A	A	A	F	A	A	A	A	A	A	A	A	A	F	A	U	A	A	A	F	F	A	Stopgain	HGSC
14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NA	CCC
15	A	A	A	A	A	U	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	F	A	A	A	A	F	A	A	F	A	F	A	A	F	F	A	A	A	A	A	Stopgain	HGSC
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	NA	CCC
17	N	N	N	N	N	N	U	N	N	N	N	U	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	Wild type	EC
18	N	N	N	N	U	N	N	F	N	F	N	N	N	N	N	A	N	F	N	N	N	N	N	F	N	F	N	F	N	N	F	N	F	A	N	F	F	N	F	N	N	N	Wild type	CCC
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	Wild type	EC
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	NA	CCC
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	NA	LGSC
22	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	F	A	A	A	A	A	A	A	Stopgain	HGSC
23	A	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	Indel	HGSC
24	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	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	Indel	HGSC
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	F	N	N	F	N	F	N	N	N	N	F	N	N	N	N	N	N	N	N	N	Wild type	EC
26	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	Missense	HGSC
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	NA	CCC
28	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	Missense	HGSC
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	N	N	F	N	N	N	N	N	N	N	N	N	F	N	N	N	F	N	N	N	Wild type	EC
30	U	U	U	U	U	U	U	F	N	U	U	U	N	N	U	U	U	U	N	U	U	N	U	N	U	U	U	U	U	U	U	U	U	U	U	N	U	U	U	A	A	Missense	HGSC
31	N	N	F	F	N	U	F	N	F	F	F	N	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	U	F	F	F	N	F	F	F	F	U	F	F	F	N	N	NA	CCC
32	U	U	U	A	U	U	U	F	A	U	U	A	A	A	U	U	F	U	A	U	U	N	A	F	U	N	A	U	U	U	U	U	A	N	A	U	N	U	U	N	U	A	Stopgain	HGSC
33	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NA	MC
34	A	A	A	A	A	A	U	A	A	A	A	A	A	F	F	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	Stopgain	HGSC
35	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NA	CCC
36	U	U	U	U	U	U	U	F	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	N	NA	LGSC
37	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	A	N	N	N	F	N	N	A	N	F	N	N	F	N	F	N	N	N	N	N	A	N	A	Missense	HGSC
38	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	N	N	F	N	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	wild type	CCC
39	U	U	U	U	A	U	U	U	U	A	U	U	U	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	U	A	A	A	A	A	A	A	A	Missense	HGSC
40	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	N	F	N	N	N	N	N	N	F	N	N	N	N	N	N	N	F	N	N	N	N	N	F	N	N	N	Wild type	EC
41	N	N	N	N	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	Wild type	EC
42	A	A	A	A	A	A	U	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	Indel	HGSC

N Normal A Abnormal ("all"/strong positive staining) A Abnormal ("nothing"/absent expression)

F Uninterpretable (no internal control) U Uninterpretable (no tumor or core)

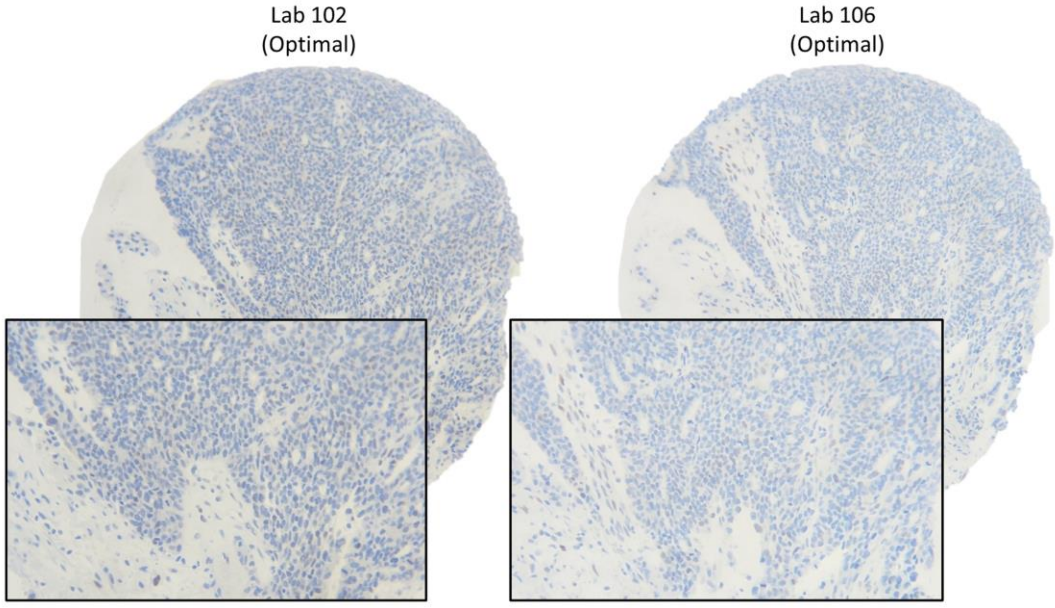

Figure 1. Representative images of very faint positive staining in tumour cell nuclei that is of much weaker intensity than staining in internal controls (Lab 102) and should be interpreted as abnormal/absent expression.

Detailed guidelines on interpretation of p53 immunostaining were recently published by the British Association of Gynecological Pathologists and have been attached at the end of this summary. Supplementary Table 1 summarizing reported staining protocol details and Supplementary Table 2 containing descriptive statistics 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 caution. 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 working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes for external quality assurance services.

Table S1. Reported p53 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	EnV FlexTRS, HIgh PH	30 min	DO-7	1:500	Dako	20050705	20 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	DO-7	Predilute (50%)	DAKO	20065933	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
103	CC1	36	Bp53-11	Pre	Ventana	F20105	32	DAB	No	Copper	DAB Ultraview
106	HIGH Ph 97C	30MIN	DO-7	PRE DILUTE	AGILENT/DAKO	20072329	30 MIN	FLEX	N	Y	DAB
107	Dako FLEX TRS High pH	30	DO-7	RTU	Dako	20069560	20	Dako FLEX	N	N	DAB
110	DAKO PT High ph 9.0@97 C	20 min	DO-7	1:800	DAKO	20050705	30 min	Dako Envision Flex	N	N	DAB
112	Bond Epitope Retrieval 2	30 minutes	DO-7	RTU	Leica	65828	15 minutes	BOND polymer refine detection	no	no	DAB
113	High pH	30	D0-7	Pre-dilute	DAKO/Agilent	20061758	15	DAKO Envision Flex HRP	N	N	DAB
114	Envision Flex TRS, High pH	30	DO-7	RTU	Dako	20061758	20	Envision FLEX DAKO Omnis	Y	N	Envision Flex DAB
120	HIER	56 MINS	Bp53-11	RTU	VENTANA	F09756	16 MINS	OPTIVIEW	N	N	DAB
125	HIER	30	DO-7	RTU	Dako	20061758	30	Envision Flex	Y	N	Dab
127	HIER	52 MIN	DO7	PREDILUTE	VENTANA	F06571	36 MIN	ULTRAVIEW DAB	Y	Y	DA
128	Ultra CC1	20 min	BP53-11	Ready to Use	Ventana/Roche	E28135	16min	Ultraview Universal DAB	No	No	DAB
132	High pH	20	DO-7	RTU	Dako	20065933	30	Envision flex	N	N	DAB
141	HIER	20	DO-7	1:800	Agilent/Dako	20050705	30	Polymer	N	N	DAB
144	Envision High Flex	20 min	DO-7	RTU	Dako	20061758	20 min	EnFlex Dako Omnis	no	no	DAB
147	ER 2 PH 8	20	DO-7	1500	DAKO	20042567	15	LEICA REFINE POL	N	N	DAB
148	CC1	16	D07	RTU	Ventana	F13475	12 min	OPTIVIEW	NO	NO	DAB
149	high pH OMNIS	20 min at 97 C	DO-7	RTU	Dako Agilent	20061758	26	EnVision Flex OMNIS	Yes	No	DAB
151	BUFFER 9.0	20MIN	D0-7	1:1500	DAKO	20027915	15MIN	BOND REFINE	N	N	DAB
159	Flex TRS High	40 min.	DO-7	RTU	Dako, Agilent	20061747	30	Dako Flex	N	N	DAB
160	CC1	48MIN	D07	1/2000	DAKO	20057047	32MIN	optiview	N	Y	DAB
168	HIER	48	DO-7	RTU	Agilent/Dako	20070843	20	Envision Flex+	N	N	DAB
175	HIER	32 min	D07	pre-dilute	Roche	F18298	32 min	OPTI-DAB (multimer)	N	Y (copper)	DAB
176	CC1	32	BP 53-11	Predilute	Ventana	E19883	40	Optiview	n	n	DAB
183	ULTRA CC1	52	DO7	RTU	VENTANA	E28136	36	ULTRAVIEW	N	N	DAB
186	HIER	20	DO-7	1:1000	Dako	20050705	15	BOND POLYMER REFINE DETECTION	N	N	DAB
192	Ultra CC1	36 minutes	Bp53-11	Ready to use	Ventana/Roche	F20105	12 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	ER2 (pH 9)	20	DO-7	RTU	LEICA	66044	15	REFINE	N	N	DAB
198	High pH HIER	30 min	DO-7	1/50	Dako/Agilent	20071295	35 min	Envision Flex/HRP	N	N	DAB
202	HIER PH9.0	10	D0-7	RTU	LEICA	65828	15	BOND POLYMER REFINE DETECTION	N	N	DAB
207	on line-Envision High PH	30	DO-7	prediluted	Dako	20069560	10	DAB Envision Flex	Y	N	DAB
209	HIER	97	DO-7	Pre-diluted	Dako	20070843	30	Polymerase	no	no	BAB
217	hier ventana cc1	48	DO-7	2000	dako	63444	32	optiview	n	y	dab
220	HIER	16	BP-53-11	PRE DILUTE	VENTANA	F01537	8	VENTANA OPTIVIEW	N	Y	DAB
228	HIER in Bond Epitope Retrieval 2	20	DO7	1:2000	AGILENT (DAKO)	20063866	15	LEICA REFINE DETECTION KIT	N	N	DAB
230	HIER	32	BP-53	predilute	Roche	F09756	32	Optiview	N	N	DAB
231	ULTRA CC1	56	DO-7	PRE-DILUTE	ROCHE/VENTANA	E28136	44	OPTIVIEW	N	N	DAB
234	Omnis/High	30	DO-7	RTU	Agilent	20069560	20	Envision Flex	N	N	DAB
236	CC1	64	BP-53-11	RTU	Roche	F15781	24	UltraView	N	N	DAB

Table S2. Descriptive statistics for p53 IHC based on CPQA assessment. Cores 1, 8 and 37 were excluded from analysis.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	39	87.18	34	34/34 (100%)	1	1	1
102	39	89.74	35	35/35 (100%)	1	1	1
103	39	84.62	33	33/33 (100%)	1	1	1
106	39	87.18	34	34/34 (100%)	1	1	1
107	39	92.31	36	36/36 (100%)	1	1	1
109	39	76.92	30	30/30 (100%)	1	1	1
110	39	71.79	28	28/28 (100%)	1	1	1
112	39	87.18	34	34/34 (100%)	1	1	1
113	39	89.74	35	34/35 (97%)	0.93	1	0.94
114	39	89.74	35	35/35 (100%)	1	1	1
120	39	82.05	32	32/32 (100%)	1	1	1
125	39	89.74	35	35/35 (100%)	1	1	1
127	39	89.74	35	34/35 (97%)	0.93	1	0.94
128	39	79.49	31	30/31 (97%)	0.92	1	0.93
132	39	74.36	29	28/29 (97%)	0.86	1	0.9
141	39	71.79	28	28/28 (100%)	1	1	1
144	39	84.62	33	32/33 (97%)	1	0.95	0.94
147	39	87.18	34	34/34 (100%)	1	1	1
148	39	69.23	27	26/27 (96%)	0.93	1	0.93
149	39	87.18	34	34/34 (100%)	1	1	1
151	39	87.18	34	34/34 (100%)	1	1	1
159	39	92.31	36	34/36 (94%)	0.86	1	0.88
160	39	89.74	35	35/35 (100%)	1	1	1
168	39	79.49	31	30/31 (97%)	0.9	1	0.92
175	39	82.05	32	32/32 (100%)	1	1	1
176	39	89.74	35	34/35 (97%)	0.92	1	0.94
183	39	74.36	29	29/29 (100%)	1	1	1
186	39	82.05	32	32/32 (100%)	1	1	1
192	39	69.23	27	27/27 (100%)	1	1	1
194	39	89.74	35	35/35 (100%)	1	1	1
198	39	84.62	33	33/33 (100%)	1	1	1
202	39	84.62	33	33/33 (100%)	1	1	1
207	39	89.74	35	35/35 (100%)	1	1	1
209	39	66.67	26	25/26 (96%)	0.89	1	0.91
217	39	89.74	35	33/35 (94%)	0.92	0.95	0.88
220	39	74.36	29	29/29 (100%)	1	1	1
228	39	76.92	30	28/30 (93%)	0.82	1	0.85
230	39	76.92	30	30/30 (100%)	1	1	1
231	39	89.74	35	35/35 (100%)	1	1	1
234	39	79.49	31	30/31 (97%)	0.92	1	0.93
236	39	89.74	35	35/35 (100%)	1	1	1

Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	The staining was considered of the highest technical quality to allow for accurate readout of the target biomarker.	PASS
Adequate	The staining was considered to be sufficient for the purpose of accurate readout of the target biomarker.	PASS
Sub-optimal	The staining was considered to be of a quality that makes readout of the test challenging, which may lead to inaccurate readout of the target biomarker.	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]

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.