Summary Report – Run 119 p53

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Overview

p53 immunostaining is a surrogate for *TP53* mutational analysis in ovarian or endometrial carcinoma, which can be used as 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) or as an aid in the diagnosis of serous tubal intraepithelial carcinoma of the fallopian tube. Interpretation of p53 immunostaining is tripartite and more complex than for most immunomarkers, with a so called "all or nothing" staining pattern indicative of abnormal expression. Moderate to strong cytoplasmic staining without nuclear staining can also correlate with underlying mutations in *TP53*, but is very uncommon. The scoring system that laboratories were asked to apply was:

- **Abnormal (A)** – indicates either complete absence of staining or moderate to strong staining in 80% or more of tumour cell nuclei, corresponding to nonsense or missense mutations in *TP53*, respectively.
- **Normal (N)** – indicates weak to moderate staining in 1-80% of tumour cells.
- **Failed (F)** – complete absence of staining in both tumour cells and normal "internal control" cells (e.g. lymphocytes and stromal cells)
- **Unsatisfactory (U)** – technical problem that makes interpretation impossible, such as core drop off or no tumour cells present

Results

Core 6 had a splicing mutation that should theoretically result in complete absence of p53 protein but some labs showed faint staining of tumour cell nuclei that was of weaker intensity than staining of internal control cells. As such, this should be interpreted as abnormal/absent p53 staining. Absent p53 staining in Core 23 was expected due to an indel mutation but many positively stained intratumoral lymphocytes caused some labs to interpret the core as wildtype staining, which was corrected during CPQA assessment. Cores 7 and 31 had very weak staining overall and could not be interpreted (failed staining) for many participants. Core 37 also showed very weak staining (wildtype or equivocal for overexpression) and is an example of a tumor with a *TP53* mutation where p53 immunostaining does not accurately reflect the underlying mutation, which can be seen in approximately 5% of ovarian or endometrial carcinomas with a *TP53* mutation. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	
106	Optimal	
107	Optimal	
110	Adequate	Weak staining
111	Optimal	Slightly weak
112	Optimal	
113	Optimal	
114	Optimal	
115	Adequate	Weak staining
120	Adequate	Weak staining
125	Optimal	
127	Optimal	Slight background in some cores
128	Adequate	Weak staining
132	Adequate	Weak staining
138	--	Slide not available for assessment
141	Adequate	Weak staining
144	Adequate	Weak staining

Lab ID	IHC Status*	Comments
147	Optimal	
149	Optimal	
151	Optimal	
159	Optimal	Slightly weak
160	Optimal	
175	Optimal	
176	Optimal	
180	Optimal	Slight background in some cores
183	Adequate	Weak staining
186	Optimal	Slightly weak
192	Adequate	Weak staining
194	Adequate	Weak staining
198	Optimal	
202	Adequate	Weak staining
207	Optimal	
220	Optimal	
228	Optimal	
230	Optimal	

*based on CPQA assessor consensus

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Garrattogram after CPQA assessment:

Lab/ Core	101	102	106	107	110	111	112	113	114	115	120	125	127	128	132	138	141	144	147	149	151	159	160	175	176	180	183	186	192	194	198	202	207	220	228	230	R1	TP53 mutation status	Histotype
1	A	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	Missense	EC
2	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	A	NA	MC
3	N	N	N	N	N	N	N	N	N	N	N	N	N	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
4	U	U	U	N	U	U	N	U	N	U	U	N	U	U	U	U	U	N	U	U	U	N	U	N	U	U	N	U	U	U	U	U	U	U	N	U	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	NA	CCC
6	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	N	A	A	Splice site	HGSC
7	N	N	N	N	N	N	N	N	N	F	F	N	N	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	F	N	F	N	N	N	N	N	Wild type	EC
8	A	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	F	A	A	A	A	A	A	A	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	NA	CCC
10	N	N	N	N	N	N	N	N	N	N	F	N	N	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	F	N	N	N	N	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	NA	HGSC
12	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	N	NA	LGSC
13	U	U	U	U	U	U	U	U	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	A	Stopgain	HGSC
14	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	N	N	N	N	N	N	N	N	N	N	N	NA	CCC
15	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	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	NA	CCC
17	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	N	N	N	N	N	N	N	N	N	N	N	Wild type	EC
18	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	N	F	N	N	N	N	F	N	F	F	N	N	N	N	N	N	N	Wild type	CCC
19	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	N	N	N	N	N	N	N	N	N	N	N	Wild type	EC
20	N	N	U	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	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	NA	LGSC
22	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	F	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	Stopgain	HGSC
23	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	Indel	HGSC
24	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	Indel	HGSC
25	N	N	N	N	N	N	N	N	N	N	N	N	N	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
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	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	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	Missense	HGSC
29	N	N	N	N	N	N	N	N	N	N	N	N	N	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
30	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
31	F	U	F	F	F	F	N	N	F	F	F	F	N	F	F	F	F	F	F	N	F	F	F	N	N	F	F	F	F	F	N	F	F	F	F	F	N	NA	CCC
32	A	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	Stopgain	HGSC
33	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	N	N	N	N	N	F	N	N	N	N	N	NA	MC
34	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	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	NA	CCC
36	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	N	NA	LGSC
37	N	N	N	N	N	N	N	N	N	N	N	N	F	N	N	F	N	N	N	N	N	N	N	N	N	N	N	N	F	F	N	F	N	N	N	N	A	Missense	HGSC
38	N	N	N	N	N	N	N	N	N	N	N	N	N	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	CCC
39	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
40	N	N	N	N	N	N	N	N	N	F	N	N	N	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	F	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	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	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	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)

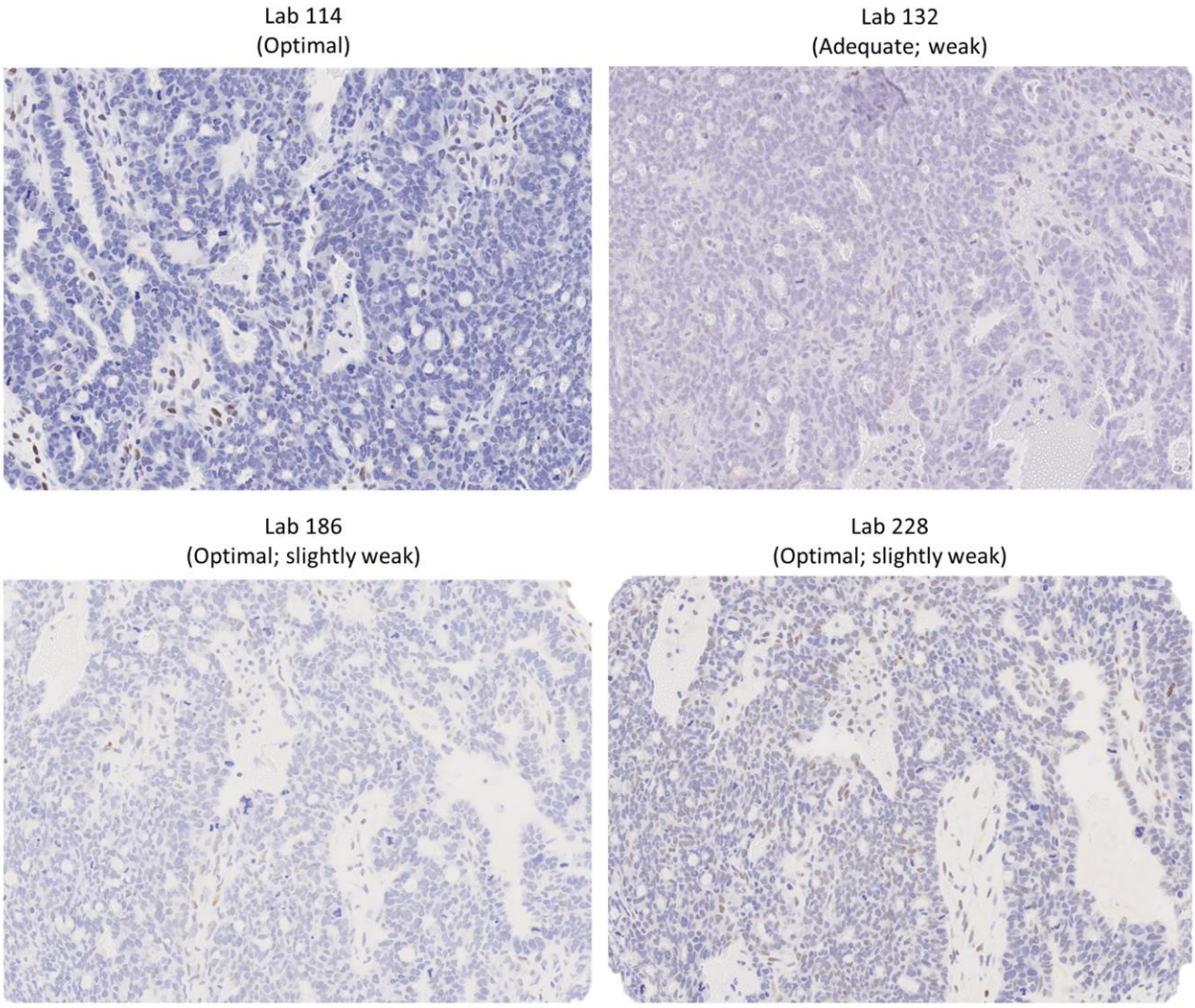

Figure 1. Representative images of p53 staining in a case with a splicing mutation that should theoretically lead to loss of p53 staining in tumour cells. Despite an identical protocol to Lab 186, Lab 228 had sufficient intensity staining in tumour cell nuclei to be considered a normal/wildtype pattern. This serves as a reminder that wild type and mutant patterns of expression can be seen in the same tumour, as well as different patterns of mutant expression in the same tumour.

As with Run 108 p53, we refer you to detailed guidelines on interpretation of p53 immunostaining 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 for p53 IHC, 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.

This report has been updated with scanned images that 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 p53 IHC staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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	DO-7	1:500	DAKO	20050705	20 MIN	DAKO Envision FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retrieval solution 9.0	20	D0-7	50/50	Agilent	20070841	30" RT	Dako Envision Flex	Y	yes CuSO4
106	Dako Omnis	LDT	High pH buffer 97 deg	30	DO-7	RTU	Agilent/Dako	20069560	30	Dako Flex Plus	Yes	No
107	Dako Omnis	LDT	Dako FLEX TRS High pH	30	DO-7	RTU	Dako	200969560	20	Dako FLEX	N	N
110	DAKO Autostainer Link 48	LDT	DAKO PT High ph 9.0@97 C	20 min	DO-7	1:800	DAKO	20050705	30 min	Dako Envision Flex	N	N
111	ULTRA BENCHMARK	commercial	HIER	48	DO-7	900	CELLMARQUE	46266	32	OPTIVIEW	N	Y
112	BOND III	LDT	BOND epitope retrieval 2 pH 9.0	30 min	DO-7	RTU	Leica	67427	15 min	BOND polymer refine detection	No	No
113	Dako Omnis	LDT	High pH	30 min	DO-7	Pre-dil	Dako	20069560	25 min	Dako Envision Flex HRP	N	N
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	D0-7	RTU	Dako	20069560	20	Envision FLEX DAKO Omnis	Y	N
115	Dako Omnis	LDT	Envision Flex High PH	30 min	DO-7	RTU	Dako	20069560	20 min	Envision Flex	N	N
120	Autostainer Link48	Commercial	HIER Waterbath	20	DO-7	RTU	Dako	20070843	30	Dako Envision Flex	N	N
125	Dako Omnis	LDT	HIER	30	DO-7	RTU	DAKO	20069560	30	EnVision Flex	Y	N
127	BENCHMARK ULTRA	LDT	HIER	52 MIN	DO7	PREDILUTE	VENTANA	F27353	36 MIN	ULTRAVIEW DAB	Y	Y
128	Benchmark Ultra	LDT	CC1	20 min	BP53-11	Ready to Use	Ventana/Roche	E28135	16min	Ultraview Universal DAB	No	No
132	Dako Autostainer	Commercial	High pH	20	DO-7	RTU	Dako	20065933	30	Envision flex	N	N
138	Dako OMNIS	LDT	EDTA HIER	20	DO-7	RTU	Dako	2006560	20	Polymer/Dako	y	N
141	Autostainer Link48	LDT	HIER	10	D0-7	1:800	Dako	20050705	30	Polymer	N	N
144	Dako Omnis	LDT	HIER	30	DO-7	RTU	Dako	20079191	20	Flex	N	N
147	Leica Bond 3	LTD	ER2 High PH	20	DO-7	1500	Dako	20042567	15	Leica Refine Kit	N	N
149	Dako OMNIS	LDT	high pH OMNIS	30 min at 97C	DO-7	RTU	Dako Agilent	20061758	26	EnVision Flex OMNIS	Yes	No
151	BOND 111	COMMERCIAL	BUFFER 9.0	20MIN	DO-7	1:1500	DAKO	20027915	15MIN	BOND REFINE	N	N
159	Autostainer 48 Link	Commercial	Flex TRS High	40 min.	DO-7	RTU	Dako, Agilent	20070841	30	Dako Flex	N	N
160	BenchMark ultra	LDT	CC1	48 MIN	DO-7	1/2000	DAKO	20063866	32 MIN	OPTIVIEW	N	Y
175	Benchmark ULTRA	LDT	HIER	32 min	D0-7	Pre-dilute	Roche	F27353	32 min	Opti-DAB	N	Y
176	Ventana Ultra	Commercial	CC1	32	BP 53-11	Predilute	Ventana	E19883	40	Optiview	N	N
180	Ventana - Roche	LDT	CC1	16 min	Bp53-11	RTU	Ventana	F27098	24 min	Optiview	N	Y
183	Ventana Benchmark	LDT	ULTRA CC1	52	DO7	RTU	VENTANA	G02444	36	ULTRAVIEW	N	N
186	LEICA BOND III	LDT	HIER	20	DO-7	1:1000	DAKO	20050705	15	BOND POLYMER REFINE DETECTION	N	N
192	BenchMark Ultra	commercial	Ultra CC1	36 min	Bp53-11	Ready to use	Ventana/Roche	F20105	12 min	Ventana Ultraview DAB	N	Y (copper)
194	BOND III	LDT	ER2 (pH 9)	20	DO-7	RTU	Leica	67427	15	Refine (polymer)	N	N
198	Dako-Omnis	LDT	HIER	30	DO-7	1/50	Dako	20071295	35	Envision Flex	N	N
202	Leica Bond III	LDT	HIER PH 6.0	10	D0-7	RTU	Leica	66845	15	Bond polymer refine detecton kit	N	N
207	DAko-Omnis	LDT	high PH	10	DO-7	presiluted	Dako	20069560	10	EnVision Flex High pH	Y	Y
220	Ventana BenchMark Ultra	commercial	HIER	16	BP-53-11	PRE DILUTE	VENTANA	F27098	8	VENTANA OPTIVIEW	N	Y
228	Bond III	commercial	HIER	20 min	DO7	1:1000	Dako/Agilent	20063866	15 min	Bond polymer detection kit	N	N
230	Benchmark Ultra	LDT	HIER	32	BP-53	predilute	Roche Diagnostics	F27098	32	OPTIVIEW	N	N

Table S2. Descriptive statistics based on CPQA assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	42	83.33	35	34/35 (97%)	0.93	1	0.94
102	42	83.33	35	34/35 (97%)	0.93	1	0.94
106	42	80.95	34	33/34 (97%)	0.93	1	0.94
107	42	85.71	36	35/36 (97%)	0.93	1	0.94
110	42	83.33	35	34/35 (97%)	0.93	1	0.94
111	42	83.33	35	34/35 (97%)	0.93	1	0.94
112	42	88.1	37	36/37 (97%)	0.93	1	0.94
113	42	83.33	35	34/35 (97%)	0.93	1	0.94
114	42	85.71	36	35/36 (97%)	0.93	1	0.94
115	42	78.57	33	32/33 (97%)	0.93	1	0.94
120	42	66.67	28	27/28 (96%)	0.93	1	0.93
125	42	85.71	36	35/36 (97%)	0.93	1	0.94
127	42	85.71	36	35/36 (97%)	0.93	1	0.94
128	42	71.43	30	30/30 (100%)	1	1	1
132	42	83.33	35	34/35 (97%)	0.93	1	0.94
138	42	78.57	33	31/33 (94%)	0.85	1	0.87
141	42	80.95	34	34/34 (100%)	1	1	1
144	42	80.95	34	33/34 (97%)	0.93	1	0.94
147	42	83.33	35	34/35 (97%)	0.93	1	0.94
149	42	85.71	36	35/36 (97%)	0.93	1	0.94
151	42	83.33	35	34/35 (97%)	0.93	1	0.94
159	42	83.33	35	34/35 (97%)	0.93	1	0.94
160	42	83.33	35	34/35 (97%)	0.93	1	0.94
175	42	88.1	37	36/37 (97%)	0.93	1	0.94
176	42	85.71	36	35/36 (97%)	0.93	1	0.94
180	42	83.33	35	34/35 (97%)	0.93	1	0.94
183	42	83.33	35	34/35 (97%)	0.93	1	0.94
186	42	83.33	35	34/35 (97%)	0.93	1	0.94
192	42	71.43	30	30/30 (100%)	1	1	1
194	42	71.43	30	30/30 (100%)	1	1	1
198	42	85.71	36	35/36 (97%)	0.93	1	0.94
202	42	73.81	31	31/31 (100%)	1	1	1
207	42	83.33	35	34/35 (97%)	0.93	1	0.94
220	42	80.95	34	33/34 (97%)	0.93	1	0.94
228	42	85.71	36	34/36 (94%)	0.87	1	0.88
230	42	83.33	35	34/35 (97%)	0.93	1	0.94

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.