Assessors' report for cIQc Run 68: p53

Assessors: B Gilks, J Garratt, J Won (recorder)

Assessment performed on Friday, May 26, 2017, at Vancouver General Hospital, BC, Canada

Overview

Interpretation of p53 is tripartite, with either complete absence of staining or strong nuclear staining in at least 80% of cells having evidence of p53 genetic abnormality (so called **"all or nothing" staining** pattern). Normal p53 wildtype staining is 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 p53 (for example, basal keratinocytes of normal skin show variable p53 positivity while the mitotically inactive superficial keratinocytes are negative). Unlike previous cIQc p53 challenges (Run 42 and 54), self-assessments were done much more consistently according to the "all or nothing" scoring system described above.

Large portions of Cores 6 and 23 were comprised of lymphocytes that were mis-interpreted during self-assessments as positive tumour cell staining. As noted previously, Core 6 is an unusual tumour, in that it has a splicing mutation that should theoretically result in complete absence of p53 protein but many labs show convincing wild type pattern. This case should be disregarded when optimizing the assay.

Consistent with Run 42 and 54 observations, some labs continued to have staining that was sufficiently weak as to make it difficult or impossible to distinguish between low-level wildtype and complete absence of p53 expression. Conversely, some labs had significant overstaining issues that made interpretation challenging and led to several incorrect scores.

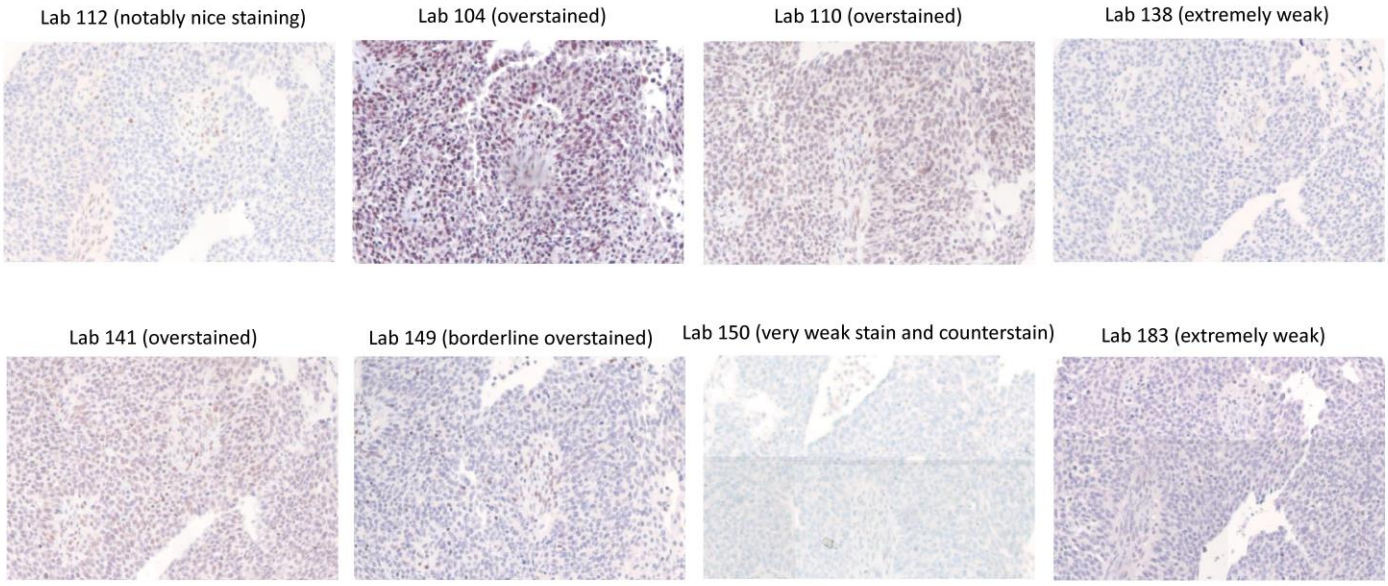

Figure 1. Representative images of staining in Core 23 (an abnormal "nothing"/absent expression case) from select labs with noted staining issues.

Participant-specific feedback for p53 IHC is summarized below:

Lab ID	IHC Status*	Assessor comments
101	Optimal	
102	Optimal	
103	Optimal	Slightly weaker staining compared to other labs
104	Failed	Overstained making interpretation difficult and very strong counterstain
106	Optimal	
107	Optimal	
110	Failed	Overstained making interpretation difficult
111	Optimal	
112	Optimal	Notably nice staining
114	--	No slide
115	Optimal	
120	Optimal	
123	Optimal	Slightly more intense staining compared to other labs
124	Adequate	Intense staining; many self-assessment scores altered during cIQc assessment (potential interpretive issue?)
125	Optimal	
126	Optimal	Slightly weaker staining compared to other labs
127	Optimal	
132	Optimal	Slightly weaker staining compared to other labs
133	Optimal	
138	Failed	Extremely weak staining; internal controls generally very faint
141	Failed	Overstained making interpretation difficult
144	Optimal	
145	Optimal	
147	Optimal	Generally more intense staining compared to other labs
148	Optimal	
149	Sub-optimal	Borderline overstained
150	Sub-optimal	Very weak staining; internal controls generally faint; very faint counterstain
155	Optimal	
159	Optimal	Very strong counterstain
160	Optimal	
168	Optimal	
176	Optimal	
183	Failed	Extremely weak staining that is difficult to distinguish from counterstain
186	Optimal	
202	Adequate	Weak staining leading to more "U" due to lack of internal control
209	Optimal	
217	--	No slide
228	Optimal	
231	Optimal	

*Based on assessment team consensus

In the Garrattograms in Figure 2, the p53 abnormal results from cIQc assessment were colour coded with pink **for "nothing"/absent expression** and red **for "all"/strong positive staining**. Uninterpretable results were also color coded with **yellow for complete absence of staining in both tumour cells and normal "internal control" cells (i.e. lymphocytes or stromal cells)** and blue **for cases where the core either came off the slide or there was no tumour in the tissue core.** Supplementary Table 1 summarizing reported staining protocol details and Supplementary Table 2 summarizing sensitivity, specificity, and Cohen's kappa for each participating laboratory can be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with caution. 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.

Figure 2. Garrattograms after cIQc assessment and self-assessment of p53 IHC.

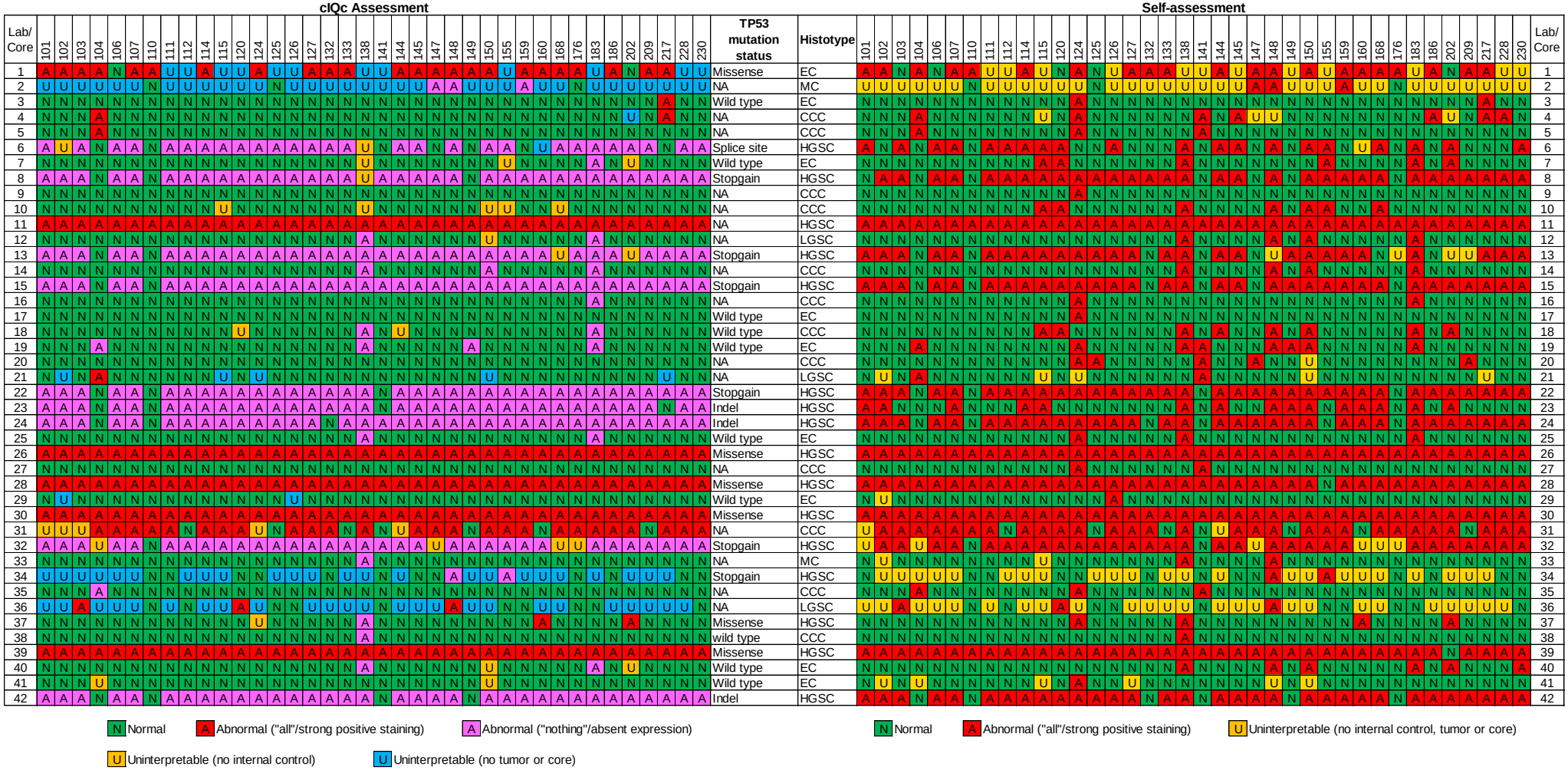

Table S1. Reported p53 staining protocols.

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	CC1	32	DO-7	1:800	DAKO	63444	32	OptiView	N	Y	DAB
102	DAKO PT HIGH pH	10/20/10	DO7	Predilute	DAKO	20031624	30 @ RT	DAKO ENVISION FLEX	NO	CUSO4	DAB+
103	CC1	36 MINS	Bp53-11	PRE	Ventana	G05267	32 mins	ULTRA VIEW	DAB ultra view	Y	DAB
104	HIER	20	DO-7	RTU	Dako	20023686	20	polymer	yes	no	DAB
106	Microwave pressure cooker	10 minutes	DO-7	1:100	Biocare	21012	30 minutes	MACH4	no	no	DAB
107	cc1	24	DO-7	1:100	Dako M7001	20027916	32	Optiview DAB	N	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	DO-7	1:800	DAKO	20023361	30 min	Dako Envision Flex	N	N	DAB
111	CC1	48 MIN	DO-7	1/900	CELL MARQUE	1510704D	32 MIN	OPTIVIEW	N	Y	DAB
112	BOND Epitope Retrieval 2	30 minutes	DO-7	RTU	LECIA	46247	15 minutes	BOND Polyner Refine Detection	no	no	DAB
114	CC1	32	DO-7	1/400	Dako	20007331	16	Optiview	N	Y	DAB
115	EnVision Flex TRS, High Ph	30 min	DO-7	RTU	Dako	20027956	20 mins	envision flex	N	N	DAB
120	Waterbath	20	DO-7	pre-dilute	Dako	20019270	20	EnVision Flex Autostainer link48	n	n	DAB
124	CC1	32 min	D0-7	PrÃ©-diluÃ©	Ventana	G03865	24	Optiview	o	n	dab
125	HIER-HIGH PH	30	D07	RTU	Dako	20030015	30	Polymer	N	N	DAB
126	Microwave Pressure Cooker, Citrate Buffer, pH 6.0	35 Minutes	DO-7	1:1000	Dako	2007332	30 minutes	Dako Envision Plus	No	No	DAB Plus
127	AUTOMATED (ULTRA CELL CONDITIONER 1)	52 MINUTES	DO7	PREDILUTE	VENTANA	G00132	36 MINUTES	ULTRAVIEW DAB	Y	N	DAB
132	high pH-Dako Flex	20	DO-7	RTU	Dako	20023687	20	Dako Envison Flex	n	n	DAB
133	HIER	64 minutes	D07	predilute	Roche	G00132	32 minutes	polymer- Optiview	n	n	dab
138	FLEX TRS High	20 (min)	DO-7	RTU	Dako	20031624	20 (min)	Envision FLEX+	N	N	FLEX DAB + sub-chromo
141	HIER	20 min	D0-7	1:800	DAKO	20023361	30 min	Polymer	no	no	DAB
144	CC1	16 min.	Bp53-11	Pre-Dilute	Ventana	G00129	16 min.	Optiview	No	Yes, copper	DAB
145	AUTOMATED HIER CC1	32	D07	1/30	CELL MARQUE	20079	28	VENTANA XT OPTIVIEW ihc v4	N	N	DAB
147	HIER ph9	20	DO-7	1:1500	DAKO	20011629	15	Polymer (Leica Refine)	n	n	DAB
148	CC1	64 MIN	DO-7	RTU	Ventana	G02415	24MIN	ULTRAVIEW DAB	N	N	DAB
149	PT Link high pH	20 min at 97 C	D07	RTU	Dako IR616	20023686	20	EnVision Flex	Yes	No	DAB
150	cc1	60	D0-7	r/u	Roche	E10278	32	ultraview	n	cu	DAB
155	CC1	30	Bp53-11	PrÃ©-diluÃ©	Ventana	E08216	16	Ultraview Dab	n	n	Dab
159	high pH	40	DO-7	pre-diluted	Dako (Agilent)	20031624	30	Flex	N	N	DAB
160	CC1	64 MIN	DO-7	1-100	DAKO	20027916	32 MIN	ULTRA-VIEW	N	COPPER CUSO4	DAB
168	HIER	48	DO-7	RTU	Dako	20031624	20	Envision Flex +	N	N	DAB
176	CC1	32	Bp 53-11	Predilute	Ventana	F08699	40	OptiView	N	N	DAB
183	Ultra CC1	64 min	p53(D07)	predilute	Roche	F05384	16 min	Ultraview	n	n	DAB
186	HIER	20 MIN	DO-7	1:1000	DAKO	20023361	15 MIN	LEICA POLYMER	N	N	DAB
202	HIER citrate pH 1	10	d0-7	RTU	Leica	47908	15	Leica Refine detection kit	no	no	DAB
209	HIER	20 mins at 97C and then cooling down to 85C for another 20mins	DO-7	Pre dilute	Dako	20019270	30mins	Envision plus	N	N	DAB
217	HIER CC1	64	DO-7	1:200	Dako	37086	36	Ultraview	N	Y	DAB
228	HIER in EDTA bufferr	20 min	DO7	1:2000	Dako	2007332	15 min	Bond refine detection system	None	None	DAB
230	HIER	60 MINS	ANTI-p53	NONE	Ventana	G00129	32 mins	iView	N	N	DAB

Table S4. Descriptive statistics for p53 after cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	42	90.48	38	37/38 (97%)	0.94	1	0.95
102	42	83.33	35	34/35 (97%)	0.93	1	0.94
103	42	92.86	39	37/39 (95%)	0.94	0.96	0.89
104	42	88.1	37	22/37 (59%)	0.4	0.73	0.13
106	42	92.86	39	36/39 (92%)	0.88	0.96	0.84
107	42	92.86	39	37/39 (95%)	0.94	0.96	0.89
110	42	100	42	29/42 (69%)	0.33	0.96	0.32
111	42	92.86	39	36/39 (92%)	0.88	0.96	0.84
112	42	92.86	39	38/39 (97%)	0.93	1	0.95
114	42	92.86	39	37/39 (95%)	0.94	0.96	0.89
115	42	85.71	36	34/36 (94%)	0.93	0.95	0.89
120	42	92.86	39	35/39 (90%)	0.88	0.91	0.79
124	42	88.1	37	36/37 (97%)	0.94	1	0.94
125	42	95.24	40	38/40 (95%)	0.88	1	0.89
126	42	90.48	38	36/38 (95%)	0.93	0.96	0.89
127	42	92.86	39	37/39 (95%)	0.94	0.96	0.89
132	42	95.24	40	36/40 (90%)	0.82	0.96	0.79
133	42	92.86	39	38/39 (97%)	0.94	1	0.95
138	42	80.95	34	25/34 (74%)	1	0.57	0.53
141	42	95.24	40	34/40 (85%)	0.63	1	0.67
144	42	88.1	37	36/37 (97%)	0.94	1	0.94
145	42	95.24	40	37/40 (93%)	0.88	0.96	0.85
147	42	95.24	40	36/40 (90%)	0.82	0.96	0.79
148	42	100	42	39/42 (93%)	0.94	0.92	0.86
149	42	92.86	39	34/39 (87%)	0.75	0.96	0.73
150	42	80.95	34	31/34 (91%)	0.94	0.89	0.82
155	42	90.48	38	36/38 (95%)	0.94	0.95	0.89
159	42	97.62	41	38/41 (93%)	0.88	0.96	0.85
160	42	90.48	38	38/38 (100%)	1	1	1
168	42	85.71	36	34/36 (94%)	0.93	0.95	0.88
176	42	97.62	41	37/41 (90%)	0.82	0.96	0.8
183	42	92.86	39	29/39 (74%)	0.93	0.63	0.51
186	42	95.24	40	37/40 (93%)	0.88	0.96	0.85
202	42	83.33	35	33/35 (94%)	0.93	0.95	0.88
209	42	92.86	39	38/39 (97%)	0.94	1	0.95
217	42	90.48	38	32/38 (84%)	0.81	0.86	0.68
228	42	92.86	39	36/39 (92%)	0.88	0.96	0.84
230	42	95.24	40	37/40 (93%)	0.88	0.96	0.84