Assessors' report for cIQc Run 54: p53 (October 2015)

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Assessment performed on Monday February 22, 2016, at Vancouver General Hospital, BC, Canada

Overview

Using the same tissue microarray as cIQc Run 42, where we tested p53/WT1/NapsinA, cIQc Run 54 was a proficiency testing exercise for only p53, which shows abnormal expression in a large majority of high-grade serous carcinomas, in contrast to the normal expression seen in low-grade serous carcinomas. p53 has also emerged as a useful marker in the diagnosis of serous tubal intraepithelial carcinoma of the fallopian tube and serous carcinoma of endometrium. Despite its increasing use in practice, there remain challenges in selection of controls and in the interpretation of p53 immunostaining, which we hoped to highlight with this run. Based on Run 42, WT1 and NapsinA staining were of sufficient quality that the decision was made to not include them in this run.

The cases used to build the tissue microarray used for this challenge were from the Coeur project, based at the University of Montreal and supported by the Terry Fox Research Institute. These cases have been subjected to detailed characterization, including p53 mutational analysis in some cases, so that histotype is well established for these cases, and the histotype diagnosis/mutation status (if known) is provided in the Garrattograms (Supplementary Figure 1). Assessors blindly reviewed all cores for all slides. Results from both self-assessment and cIQc assessment are provided below.

p53 IHC interpretation

Interpretation of p53 is tripartite, with either complete absence of staining or strong nuclear staining in at least 80% (usually of cells being 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 tumors/tissues with wildtype p53 (for example, basal keratinocytes of normal skin show variable p53 positivity while the mitotically inactive superficial keratinocytes are negative). Similar to Run 42, at the time of assessment it became apparent that interpretation for self-assessments was not done consistently, according to the all or nothing guidelines noted above. All available slides were then re-scored by the assessment team.

Consistent with Run 42 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. In Supplementary Figure 1, we color coded the p53 abnormal results in the Garrattogram with **pink for "nothing"/absent expression** and **red for "all"/strong positive staining**. Uninterpretable results include a mix of cases where the core came off the slide, there is no tumor in the tissue core, or there was complete absence of staining (in both tumor cells and normal "internal control" cells).

Internal control and on-slide control tissues

It is critically important that diagnosis of complete absence of p53 staining in tumor cells only be made when there is a positive internal control (e.g. lymphocytes or stromal cells) present. This is the same situation as for interpretation of MMR staining. In Run 54, for example cores 2, 12, and 15 were most commonly considered uninterpretable; although there was no staining of tumor cells, there was not a convincing internal control. The small cores used on this array limit the amount of normal tissue to reliably assess the internal control staining. A 1-mm core tissue microarray is currently being constructed and will be used for future p53 challenges. Nevertheless, *labs with many uninterpretable cores are encouraged to increase their staining intensity to render*

the internal control cells positive. With regards to on-slide positive control samples, we recommend the use of normal tonsil, which should show heterogeneous p53 expression in the germinal center and basal layer of squamous epithelium with weak to moderate intensity (occasional cells with strong staining can be also seen). If you are using a multi-tissue block for your on-slide control, the common 4-tissue combination of liver, pancreas, tonsil and colon/appendix works well, with p53 staining seen in tonsil and colonic crypts while other tissues are negative.

Case 6 is an unusual tumor, 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. Case 27 is another challenging case, in which the central assessment changed some abnormal to normal by attempting to apply a similar threshold to all tissue microarrays. However, this case may have antigen preservation issues.

Participant-specific feedback for p53 IHC is summarized below:

Lab ID	Assessor comments
102	
103	
104	
106	
107	
109	
110	Background blush observed
111	
112	Particularly nice staining noted by assessors; very clean and easy to interpret
113	Very strong counterstain; aggressive pretreatment.
114	
116	Overall weak staining compared to other labs
119	Overall weak staining compared to other labs
122	
124	Generally weak staining compared to other labs
125	
126	General lack of internal controls, leading to many uninterpretable results considered "Unsatisfactory"
127	
132	
133	Specific but general lack of sensitivity.
139	
141	
144	
145	Generally weak staining compared to other labs
147	
148	General lack of internal controls, leading to many uninterpretable results considered "Unsatisfactory"
149	
152	Overall lack of internal controls, leading to many uninterpretable results considered "Unsatisfactory"; very weak staining intensity

153	
157	
159	Occasional core lacking internal controls, leading to more uninterpretable results
160	Weak staining in internal controls
161	Weak staining in internal controls
162	Particularly nice staining noted by assessors
164	Weak staining and weak internal controls
165	Tissue appears to have undergone strong pretreatment
167	Weak staining and weak internal controls
168	
170	Weak staining and weak internal controls
176	Weak staining and weak internal controls
177	Provided tonsil control has very nice weak to moderate intensity staining.
182	Slightly weak staining intensity, but still good contrast due to weak counterstain
189	Almost complete lack of internal controls, leading to many uninterpretable results; extremely weak staining intensity
191	
193	
198	
202	
207	
209	
212	Weak staining and weak internal controls; provided tonsil control has very weak staining
221	Possible issue with dispensing of primary antibody on a portion of the slide

Garrattograms after self-assessment and cIQc assessment of p53 staining are presented in Supplementary Figure 1. Supplementary Table 1 summarizing reported staining protocol details and 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.

Figure S1. Garrattograms after p53 self-assessment and cIQc assessment.

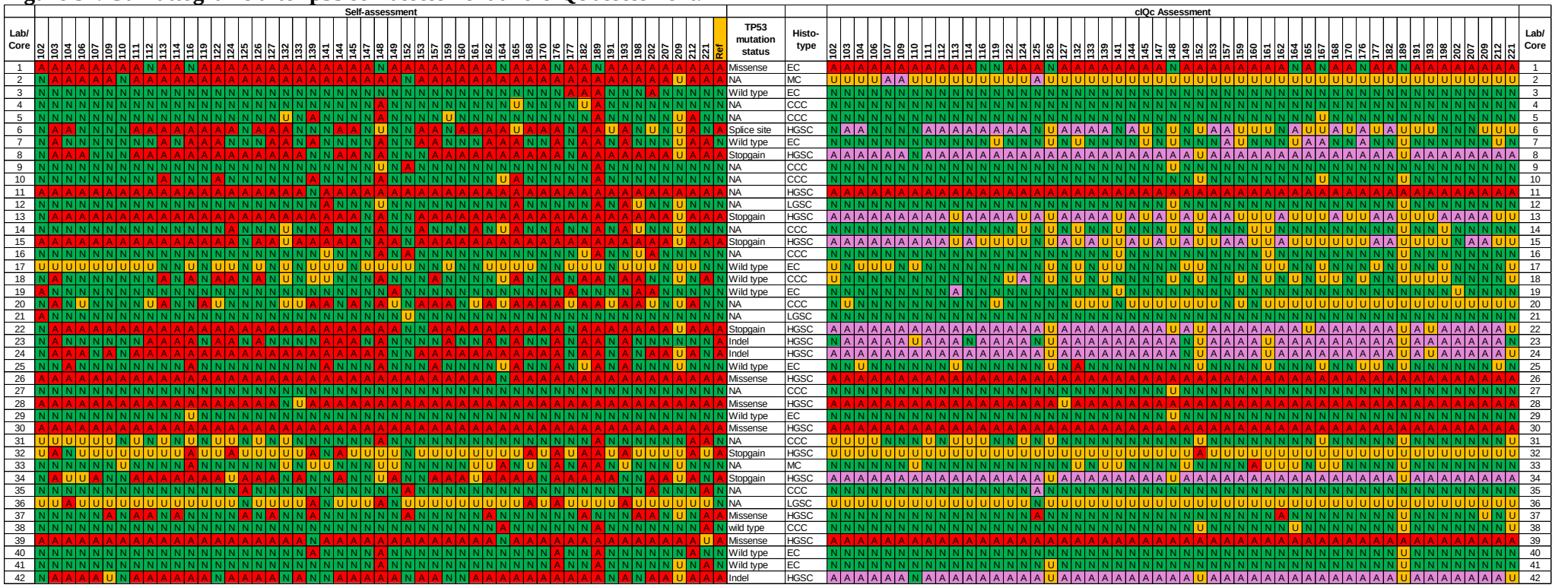

Table S1. Reported p53 staining protocols.

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
102	Dako 3in1 High pH	10/20/10	DO-7	1:75	Dako	20007332	30" RT	DAKO FLEX	NO	CUSO4	DAB+
103	CC1	36 mins	Bp53-11	predilute	Ventana	F03823	32 mins	Ventana Ultra	N	N	DAB
104	HIER	20	PAB 1801	1/1000	Callbiochem	OD119094	30	DAKO EnVision Flex	yes	no	EnVision Flex DAB
106	Microwave pressure cooker	10 minutes	DO-7	1:100	Biocare	7113	30 minutes	MACH4	no	no	DAB
107	cc1	24 min	DO-7	1:100	Dako	2007331	32	Optiview DAB	N	Y	DAB
109	HIER CC1 HIGH pH	56 MIN	Bp53-11	RTU	VENTANA	E10069	16 MIN	OPTIVIEW	N	YES COPPER	DAB
110	PT High pH 9.0	20 min @ 97C	D0-7	1:800	DAKO	20023361	30 min	Dako Flex HRP	N	N	DAB
111	CC1	48 min	DO-7	1/900	Cell Marque	1510704C	32 min	Optiview	n	Copper	DAB
112	Bond Epitope Retrieval 2	30 minutes	DO-7	RTU	Leica	41536	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
113	High pH Target Retrieval Buffer	30'	DO-7	1/2000	Dako	95381	15'	Flex+	yes	no	DAB
114	CC1	32	DO-7	1/400	Dako	20007331	16	Optiview	N	Copper	DAB
116	CC1	std -60 min	D07	1/100	Cell Marque	4307C	32 min	ultraView DAB	N	.	DAB
119	HIER	30 min	Bp53-11	predilute	Ventana	F03823	32 min	Ultraview	no	no	DAB
122	ER2-20	20 min	DO-7	RTU	Leica/Nova Castra	25342	15 min	Polymer refine	N	N	DAB
124	CC1	60	DO7	predilute	Venana	F05384	32	Ultra View	n	n	Dab
125	ER2-20	20 min	DO-07	1/5000	DAKO	20023361	15 min	Bond Polymer Refine	n	y	DAB
126	microwave pressure cooker, with citrate buffer ph 6.01	35 minutes	D0-7	1:1500	DAKO	20007332	30 minutes	Envision + Mouse	N	N	DAB
127	Ultra CC1	52	D0-7	Predilute	Ventana	E10278	36	Ultraview DAB	Y	N	DAB
132	Envision Flex High pH	20	DO-7	RTU	Dako	20014495	20	Envision Flex	n	n	DAB
133	hier	64 minutes	D07	Predilute	Roche	F02150	40 minutes	Optiview	no	no	dab
139	HIER CC1 automated	60	Bp53-11	ready use	Ventana	F01700	30	Iview DAB	Y	no	DAB
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	F01700	16 min	OptiView	N	Copper	DAB
145	CC1	30	D07	1/30	CELL MARQUE	20078	24	XT ULTRAVIEW DABv3	N	N	DAB
147	HIER, pH9-EDTA (ER2)	30	DO-7	1:500	Dako	71709	15	Bond Refine Polymer	N	N	DAB
149	PT Link high pH 97 C	20 min	D07	RTU	Dako	20010414	20 min	EnVision Flex	Yes	No	DAB
152	HIER	45 minutes	Bp53-11	predilute	Roche Ventana	E05536	16 minutes	Iview	No	Yes	DAB
153	HEAT CC1	32	DO-7	n/a	Ventana/Roche	E10278	48	Optiview Dab	N	Y	Dab
157	CC1	24 MIN.	BP53-11	predilute	VENTANA	F03823	8 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
159	High pH	40 total	D0-7	predilute	DAKO	20019270	30	FLEX	no	no	DAB
160	CC1	64MN	DO-7	1:100	Dako	20023361	32 min	ULTRAVIEW	N	CUSO4	DAB
161	HIER-High EDTA TRIS	20 Minutes	DO-7	RTU	DAKO	20019270	20 Minutes	Envision Flex	No	No	DAB
162	CC1 Ventana	48 min	DO-7	1:2000	Dako	63444	32 min	OptiView Ventana	-	-	Optiview DAB
164	ULTRA CC1	16	DO-7	predilute	Ventana	F02150	12	Optiview	N	N	DAB
165	hier	30 min	ab-5	1/500	nÃ©o markers	186p702c	32 min	ultraview dab	n	n	dab
168	HIER	48	DO-7	RTU	Dako	20019272	20	Envision Flex +	N	Y	DAB
170	tampon edta	20	DO-7	RTU	Dako	20019270	20	flex	y	y	DAB
176	CC!	16	Bp 53-11	predilute	Ventana	E10069	20	Optiview	N	N	DAB
177	CC1	30	D0-7	1:100	Dako	20007332	32	UltraView Universal	n	n	DAB
182	HEIR	20	DO-7	RTU	Leica Biosystems	28958	15	Leica Refine Polymer	N	N	DAB
189	CC1	64	DO-7	pre-dilute	Ventana	unknown	16	iView w/A-B Block	N	N	DAB
191	CC1	30'	DO-7	1/100	DAKO	95381	24'	ultraview DAB	N	N	DAB
193	High pH buffer 9.0	30 minutes	DO-7	RTU	Dako	unknown	20 minutes	Polymer Flex+ envision	yes	no	DAB
198	Citrate ph 6.2 pressure cooker	5min	DO-7	1/200	Novocastra?Leica	6017844	30 min	MACH 1 polymer	Y	Y	DAB
202	citrate PH9.5	20 min	d0-7	rtu	leica	40638	15 min	leica refine detection kit	n	n	dab
207	CC1	76 minutes	D07	Prediluted	Ventana	F05384	32 minutes	Ultraview	Y	Y	DAB
209	HIER	20 mins at 97C and 20 mins cooling down to 85C	DO-7	Pre dilute	Dako	20010414	30	Envision +	N	N	DAB
212	Heat	30	DO-7	RTU	Dako	20019273	30	Envision Flex			
221	PTM - pH6	25 Minutes	D07	1:200	Cell Marque	1320608	30 Minutes	Mouse EnVision	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	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
102	42	85.71	36	33/36 (92%)	1	0.81	0.87	1	0.83
103	42	88.1	37	36/37 (97%)	1	0.94	0.95	1	0.94
104	42	85.71	36	35/36 (97%)	1	0.94	0.95	1	0.94
106	42	88.1	37	35/37 (95%)	1	0.88	0.91	1	0.89
107	42	92.86	39	37/39 (95%)	1	0.88	0.92	1	0.89
109	42	95.24	40	38/40 (95%)	1	0.88	0.92	1	0.9
110	42	85.71	36	32/36 (89%)	1	0.73	0.84	1	0.76
111	42	90.48	38	37/38 (97%)	1	0.94	0.96	1	0.95
112	42	92.86	39	38/39 (97%)	1	0.94	0.96	1	0.95
113	42	83.33	35	33/35 (94%)	0.95	0.93	0.95	0.93	0.88
114	42	90.48	38	36/38 (95%)	1	0.88	0.92	1	0.89
116	42	88.1	37	35/37 (95%)	1	0.87	0.92	1	0.89
119	42	85.71	36	34/36 (94%)	1	0.87	0.91	1	0.88
122	42	88.1	37	36/37 (97%)	1	0.93	0.96	1	0.94
124	42	83.33	35	33/35 (94%)	0.95	0.93	0.95	0.93	0.88
125	42	97.62	41	37/41 (90%)	0.96	0.82	0.88	0.93	0.8
126	42	57.14	24	22/24 (92%)	1	0.75	0.89	1	0.8
127	42	90.48	38	37/38 (97%)	1	0.93	0.96	1	0.94
132	42	76.19	32	30/32 (94%)	0.94	0.93	0.94	0.93	0.87
133	42	90.48	38	37/38 (97%)	1	0.94	0.96	1	0.95
139	42	80.95	34	33/34 (97%)	1	0.93	0.95	1	0.94
141	42	76.19	32	30/32 (94%)	1	0.86	0.9	1	0.87
144	42	90.48	38	37/38 (97%)	1	0.94	0.96	1	0.95
145	42	80.95	34	33/34 (97%)	1	0.92	0.95	1	0.94
147	42	90.48	38	36/38 (95%)	1	0.88	0.92	1	0.89
148	42	61.9	26	24/26 (92%)	1	0.82	0.88	1	0.84
149	42	85.71	36	32/36 (89%)	1	0.75	0.83	1	0.77
152	42	57.14	24	23/24 (96%)	1	0.89	0.94	1	0.91
153	42	88.1	37	36/37 (97%)	1	0.93	0.96	1	0.94
157	42	92.86	39	37/39 (95%)	0.96	0.94	0.96	0.94	0.89
159	42	80.95	34	33/34 (97%)	1	0.93	0.95	1	0.94
160	42	83.33	35	33/35 (94%)	0.95	0.92	0.95	0.92	0.88
161	42	64.29	27	26/27 (96%)	1	0.91	0.94	1	0.92
162	42	85.71	36	35/36 (97%)	1	0.94	0.95	1	0.94
164	42	76.19	32	30/32 (94%)	1	0.86	0.9	1	0.87
165	42	80.95	34	32/34 (94%)	0.95	0.92	0.95	0.92	0.87
167	42	66.67	28	25/28 (89%)	0.93	0.85	0.88	0.92	0.78
168	42	83.33	35	34/35 (97%)	1	0.93	0.95	1	0.94
170	42	83.33	35	34/35 (97%)	1	0.92	0.96	1	0.94
176	42	80.95	34	31/34 (91%)	0.95	0.86	0.9	0.92	0.82
177	42	83.33	35	34/35 (97%)	1	0.93	0.95	1	0.94
182	42	90.48	38	37/38 (97%)	1	0.94	0.96	1	0.95
189	42	35.71	15	14/15 (93%)	1	0.83	0.9	1	0.86
191	42	80.95	34	33/34 (97%)	1	0.92	0.95	1	0.94
193	42	76.19	32	31/32 (97%)	1	0.91	0.95	1	0.93
198	42	80.95	34	32/34 (94%)	1	0.87	0.9	1	0.88
202	42	88.1	37	34/37 (92%)	1	0.81	0.88	1	0.83
207	42	90.48	38	36/38 (95%)	1	0.88	0.92	1	0.89
209	42	85.71	36	36/36 (100%)	1	1	1	1	1
212	42	78.57	33	32/33 (97%)	1	0.92	0.95	1	0.94
221	42	64.29	27	26/27 (96%)	1	0.89	0.95	1	0.91