Run 42 Assessment Meeting

Assessor's report for CIQC Run 42: Napsin, WT1, p53

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Assessment done Monday Feb 2, 2015, at Foothills Hospital, Calgary

Background: There is increasing emphasis on accurate diagnosis of ovarian carcinoma histotypes, as this information can be used to guide patient therapy, and referral to hereditary cancer programs. For example, neoadjuvant chemotherapy is largely reserved for advanced stage high-grade serous carcinomas, with histotype diagnosis often made based on a small omental needle core biopsy where immunostaining may be required for histotype confirmation. Furthermore, patients with high-grade serous carcinoma of tube/ovary have an approximately 20% chance of having a germline BRCA mutation, while patients with endometrioid or clear cell carcinomas of ovary have a likelihood of Lynch Syndrome that is comparable to that of patients with colorectal or endometrial carcinoma, and consideration should be given in these patients to MMR testing. In CIQC Run 42 we are assessing the performance of laboratories in staining for the most commonly used markers for ovarian carcinoma histotype diagnosis, specifically WT1 (a marker of high-grade serous and low-grade serous carcinoma, typically negative in endometrioid, clear cell and mucinous carcinoma), NapsinA (a marker of clear cell carcinoma, typically negative in other histotypes) and 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. 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 is provided in the Garratograms. Below are our subjective assessments of the performance of each laboratory where slides were available for review at the time of the assessment meeting. For those cores where the assessment team changed the diagnosis from the self entered result (for example if staining was considered positive on self-assessment but on review the positivity was felt to be in benign stromal cells and not tumor cells, which were negative) the new result was entered into the Garratogram and lab performance calculated based on the results of the assessment team's interpretation.

NAPSIN A

Most laboratories had optimal staining for NapsinA, which gives granular cytoplasmic staining in clear cell carcinomas. Two of the clear cell carcinomas on the array showed weak/focal staining: core 4 showed very weak staining, which was not always appreciable on some slides, while staining of core 35 was equivocal, with rare cells staining on some slides only. We did note problems with false positive

staining (in a perinuclear distribution) in labs using the pre-dilute antibodies and those labs may wish to try an alternative primary antibody.

Lab No	NapsinA
102	optimal
103	optimal, weak staining present for core 4
107	optimal
110	optimal, true weak/equivocal staining of core 35
111	optimal
112	optimal
114	Optimal, weak staining of a few cells in core 4
116	optimal
119	Inadequate, with false positive staining (in a perinuclear distribution), for cores 1, 2, 29, and 33
124	Inadequate, with false positive staining (in a perinuclear distribution), for cores 1, 2, 29, and 33
127	Suboptimal, generally weak staining compared to other laboratories, false negative for core 4
132	Inadequate, very high background with false positive staining (in a perinuclear distribution), for cores 1, 2, 29, and 33
144	optimal
146	optimal, with weak staining of core 35
148	Suboptimal, with background staining (especially in cores 1, 2, 29 and 33, in a perinuclear distribution)
159	Suboptimal, with weak overall staining compared to other labs, and false negative in cores 4 and 9
162	optimal, core 9 scored as negative but uninterpretable on review
168	optimal
170	Suboptimal, core 11 is false positive, but it was considered likely that it was leaching into the cytoplasm from the strong positive p53 nuclear stain (the staining is not as granular as usual NapsinA staining) NB NapsinA and p53 combined as dual stain
190	Inadequate, with false positive staining (in a perinuclear distribution), for cores 1, 2, 29, and 33
191	optimal, with a few positive cells in core 35
198	optimal, with a few positive cells in core 35
199	optimal, weak staining of a few cells in core 4
202	optimal
207	optimal, weak staining of a few cells in core 4

WT1

WT1 is a nuclear stain that stains both low-grade serous and high-grade serous carcinomas of tubo-ovarian origin, as well as mesotheliomas and sex cord-stromal tumors of ovary. It occasionally stains non-serous histotypes (note that core 7, and endometrioid carcinoma, shows weak WT1 immunoreactivity). It can also be used to distinguish between a primary tubo-ovarian high-grade serous carcinoma and metastatic breast carcinoma in patients with a BRCA1/2 germline mutation and an adnexal mass. Almost all labs showed optimal staining for WT1. Cores 7 and 12 show a range from weak to moderately intense positivity. In practice there is often variable WT1 staining in a tumor, an effect that is attributable to fixation effects in most cases, and the variable staining observed for these cores may have reflected variable fixation within the cores, such that sections from different levels of the tissue microarray block showed differences in WT1 staining intensity. As with NapsinA, those labs using predilute primary antibody were more likely to have problems with their staining, specifically increased background.

Lab No	WT1
102	optimal, false positive interpretation for core 4 with staining of benign stromal cells only
103	Suboptimal, with false negative results for cores 7 and 12
104	optimal, with interpretation errors for cores 12 (false negative) and 16 (false positive). One false negative core after re-assessment (core 7)
106	optimal
107	optimal, weak positivity on core 7, based on assessment review
109	optimal
110	optimal
112	optimal, core 18 positive only in benign cells, based on assessment review
116	optimal, core 16 false positive as only benign cells staining on review, one false negative (core 7)
119	Suboptimal, accurate self assessment results but there is very high background staining, compared to other labs
124	optimal
127	Inadequate; high background and false negatives for cores 6, 7, 12 and 37
135	optimal, core 10 called positive but on assessment review only benign cells staining
144	optimal, weak positive staining of core 7 on assessment review
146	optimal
148	Optimal, weak pos staining of core 7 and cytoplasmic staining only for core 17 (i.e. interpretive errors only)
149	optimal, core 12 considered positive on review
151	optimal, core 16 considered negative in tumor cells, while core 21 considered uninterpretable
159	optimal
161	optimal, core 16 considered negative in tumor cells on review
162	optimal, on review core 7 considered equivocal, while core 36 was

	considered positive on review
168	optimal
170	optimal
191	optimal, cores 16 and 41 were considered false positive on review (only benign cells staining)
198	optimal, one false negative core (core 7)
199	optimal, core 16 considered negative on review (only benign cells staining)
202	optimal, cores 10, 29 and 41 considered negative at time of assessment review
207	optimal, with focal weak staining of core 7 at review

p53

The most commonly used anti-p53 antibody has been around for many years, but it was only relatively recently appreciated that for endometrial and tubo-ovarian carcinomas, at least, interpretation of p53 is tripartite, with either complete absence of staining or strong nuclear staining in more than 80% of cells being evidence of p53 genetic abnormality (so called **"all or nothing"** staining pattern). Normal or p53 wild type staining is 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 wild-type p53 (for example, basal keratinocytes of normal skin show p53 staining). This CIQC run is the first p53 proficiency testing run using a range of tumor samples with known p53 mutation status, to the best of our knowledge.

At the time of assessment, we immediately noticed that interpretation was not done consistently, according to the all or nothing guidelines noted above. We therefore re-scored all the slides that were available for review at the time of the assessment meeting. Having done that, we identified too patterns of problematic p53 staining: 1. the main problem identified was in those labs where the staining was sufficiently weak that it was difficult or impossible to distinguish between low level expression and complete absence of expression, 2. a few labs had too strong staining, such that it was difficult or impossible to distinguish between normal (wild-type) expression and strong staining indicative of a mutation. In the Garratogram, we color coded the p53 abnormal results with pink for "nothing" or absent expression, and red for "all" or strong positive staining.

We have not offered specific comments to each laboratory based on their p53 staining, given that this was a first attempt at proficiency testing for this marker, and that the small cores used on this array made it particularly difficult to distinguish weak focal staining from complete absence of staining. We plan to use larger cores in a future run to test performance for this marker, which is becoming increasingly relevant in practice.

P53

Lab/ Core	101	102	103	104	106	107	109	110	111	112	114	116	119	122	124	127	132	133	139	144	147	148	149	152	153	157	159	161	162	165	167	168	170	176	177	191	198	199	202	207	209	210	XXX	Gold	Histotype	TP53 mutation status
1	A	A	A	A	A	A	A	A	A	A	A	N	N	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	N	A	A	A	A	A	A	A	A	N	A	A	A	EC	Missense
2	U	U	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	U	U	A	A	A	A	A	A	A	A	A	A	A	A	U	A	MC	NA
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	N	N	N	N	N	N	N	EC	Wild type
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	U	N	N	N	N	N	N	N	N	N	N	N	N	COC	NA
5	N	N	N	A	N	N	N	N	A	N	N	A	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	A	A	U	N	N	N	N	N	A	N	N	N	N	N	COC	NA
6	N	N	A	A	N	N	N	N	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	A	A	A	A	A	A	N	A	N	A	HGSC	Splice site
7	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	A	N	N	N	A	A	N	N	N	N	N	N	A	N	N	N	N	A	N	A	A	N	N	N	N	N	N	EC	Wild type
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	N	A	A	A	A	HGSC	Stopgain
9	U	U	N	U	U	N	N	U	U	U	U	U	N	N	N	N	U	N	U	U	N	U	N	N	N	N	N	N	U	U	U	U	N	U	N	N	N	U	N	N	N	N	N	N	COC	NA
10	N	N	A	A	A	N	N	N	N	N	N	A	A	A	N	N	N	N	A	N	A	A	A	N	N	N	N	N	N	A	A	A	U	N	A	A	N	A	A	N	N	U	N	N	COC	NA
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	A	A	HGSC	NA
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	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	LGSC	NA
13	A	A	A	A	A	A	A	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	A	A	A	A	A	A	A	A	A	A	HGSC	Stopgain
14	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	A	N	A	N	N	N	A	A	N	N	N	N	N	N	A	A	U	N	A	N	N	N	N	N	N	N	N	N	COC	NA
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	A	A	HGSC	Stopgain
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	A	N	N	N	U	N	N	A	U	N	N	N	N	N	N	N	N	N	N	N	COC	NA
17	A	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	EC	Wild type
18	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	A	N	N	A	N	N	A	A	A	A	U	N	A	N	N	N	N	N	N	N	N	N	COC	Wild type
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	N	N	EC	Wild type
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	COC	NA
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	LGSC	NA
22	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	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	A	A	HGSC	Stopgain
23	A	N	A	A	N	A	A	N	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	N	A	A	A	A	A	A	N	A	A	A	A	A	A	A	N	N	A	A	A	HGSC	Indel
24	A	A	A	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	A	A	A	A	HGSC	Indel
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	EC	Wild type
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	A	A	HGSC	Missense
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	U	N	N	N	N	N	N	N	N	N	N	N	N	COC	NA
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	N	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	HGSC	Missense
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	N	N	N	N	N	N	N	EC	Wild type
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	A	A	A	A	A	A	A	HGSC	Missense
31	U	N	N	A	N	N	N	N	A	N	N	A	A	N	N	N	A	N	A	A	N	A	N	N	N	N	N	U	U	A	A	A	U	N	A	N	U	N	A	N	N	N	N	N	COC	NA
32	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	U	A	U	U	U	U	U	A	U	U	U	U	U	U	U	U	U	A	A	HGSC	Stopgain
33	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	N	N	N	N	N	N	N	N	MC	NA
34	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	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	HGSC	Stopgain
35	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	COC	NA
36	N	N	N	N	N	N	N	N	N	N	N	A	A	N	N	N	A	N	A	N	N	A	U	U	N	U	U	U	U	A	A	A	N	A	N	N	N	A	N	N	N	N	N	N	LGSC	NA
37	A	N	N	N	N	A	A	N	N	N	A	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	A	N	A	N	N	N	A	N	N	A	N	N	A	N	N	A	N	A	HGSC	Missense
38	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	A	A	N	N	N	N	N	N	A	N	U	N	N	N	N	N	N	N	N	N	N	N	COC	wild type
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	A	A	A	A	A	A	A	HGSC	Missense
40	N	N	N	N	N	N	N	N	A	N	N	A	A	N	N	N	A	N	A	A	N	N	A	A	N	N	N	N	N	N	A	A	N	N	A	N	N	N	N	N	N	N	N	N	EC	Wild type
41	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	EC	Wild type
42	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	A	A	A	A	A	A	A	A	A	N	A	A	A	A	HGSC	Indel

Key: A = Abnormal B = Normal U = Unsatisfactory

building towards

cIQc

canadian Immunohistochemistry Quality control

p53 Descriptive Statistics

Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	42	88.1	37	33/37 (89%)	0.94	0.86	0.83	0.95	0.78
102	42	92.86	39	35/39 (90%)	0.75	1	1	0.85	0.78
103	42	97.62	41	39/41 (95%)	0.94	0.96	0.94	0.96	0.9
104	42	95.24	40	36/40 (90%)	0.94	0.87	0.84	0.95	0.8
106	42	92.86	39	35/39 (90%)	0.81	0.96	0.93	0.88	0.78
107	42	97.62	41	39/41 (95%)	0.94	0.96	0.94	0.96	0.9
109	42	97.62	41	40/41 (98%)	0.94	1	1	0.96	0.95
110	42	95.24	40	37/40 (93%)	0.82	1	1	0.88	0.84
111	42	95.24	40	35/40 (88%)	0.94	0.83	0.8	0.95	0.75
112	42	95.24	40	39/40 (98%)	0.94	1	1	0.96	0.95
114	42	92.86	39	39/39 (100%)	1	1	1	1	1
116	42	95.24	40	32/40 (80%)	0.88	0.74	0.71	0.89	0.6
119	42	97.62	41	35/41 (85%)	0.88	0.83	0.79	0.91	0.7
122	42	97.62	41	39/41 (95%)	0.94	0.96	0.94	0.96	0.9
124	42	97.62	41	40/41 (98%)	0.94	1	1	0.96	0.95
127	42	97.62	41	40/41 (98%)	0.94	1	1	0.96	0.95
132	42	95.24	40	34/40 (85%)	0.94	0.78	0.76	0.95	0.7
133	42	97.62	41	41/41 (100%)	1	1	1	1	1
139	42	92.86	39	30/39 (77%)	0.88	0.7	0.67	0.89	0.54
144	42	95.24	40	37/40 (93%)	0.94	0.91	0.89	0.95	0.85
147	42	97.62	41	39/41 (95%)	0.94	0.96	0.94	0.96	0.9
148	42	95.24	40	36/40 (90%)	0.94	0.87	0.84	0.95	0.8
149	42	90.48	38	29/38 (76%)	0.94	0.62	0.67	0.93	0.54
152	42	90.48	38	31/38 (82%)	0.94	0.71	0.73	0.94	0.64
153	42	97.62	41	36/41 (88%)	0.78	0.96	0.93	0.85	0.75
157	42	95.24	40	39/40 (98%)	0.94	1	1	0.96	0.95
159	42	97.62	41	40/41 (98%)	1	0.96	0.95	1	0.95
161	42	92.86	39	38/39 (97%)	0.94	1	1	0.96	0.95
162	42	85.71	36	36/36 (100%)	1	1	1	1	1
165	42	92.86	39	34/39 (87%)	0.94	0.83	0.79	0.95	0.74
167	42	95.24	40	29/40 (73%)	0.88	0.61	0.63	0.88	0.47
168	42	95.24	40	31/40 (78%)	0.94	0.65	0.67	0.94	0.56
170	42	73.81	31	28/31 (90%)	0.82	1	1	0.82	0.81
176	42	100	42	42/42 (100%)	1	1	1	1	1
177	42	95.24	40	33/40 (83%)	0.94	0.74	0.73	0.94	0.66
191	42	97.62	41	38/41 (93%)	0.94	0.92	0.89	0.96	0.85
198	42	95.24	40	40/40 (100%)	1	1	1	1	1
199	42	97.62	41	38/41 (93%)	0.94	0.92	0.89	0.96	0.85
202	42	95.24	40	35/40 (88%)	0.94	0.83	0.8	0.95	0.75
207	42	97.62	41	41/41 (100%)	1	1	1	1	1
209	42	97.62	41	34/41 (83%)	0.59	1	1	0.77	0.63
210	42	95.24	40	38/40 (95%)	0.88	1	1	0.92	0.9

WT1

Lab/ Core	102	103	104	106	107	109	110	112	116	119	124	127	135	144	146	148	149	151	159	161	162	168	170	191	198	199	202	207	210	Histotype
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	EC
2	N	N	N	U	N	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	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	EC
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	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	CCC
6	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
7	P	N	N	P	P	P	P	P	N	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	EC
8	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
9	U	N	N	U	N	U	U	U	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	CCC
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	CCC
11	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	LGSC
12	P	N	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P	LGSC
13	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	N	N	N	CCC
15	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
16	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	N	N	N	N	CCC
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	EC
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	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	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	CCC
21	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U	U	P	P	P	P	P	P	P	P	P	P	P	LGSC
22	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
23	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
24	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	EC
26	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	CCC
28	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	EC
30	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
31	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	N	N	N	U	N	N	N	N	CCC
32	N	N	N	U	N	N	U	U	U	U	U	N	U	N	U	N	U	N	N	U	U	N	N	N	N	N	U	U	N	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	U	N	N	MC
34	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	CCC
36	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	P	P	LGSC
37	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	CCC
39	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC
40	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	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	EC
42	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	HGSC

Key: P = Positive N = Negative U = Unsatisfactory

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WT1 Descriptive Statistics

Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
102	42	97.62	41	41/41 (100%)	1	1	1	1	1
103	42	100	42	40/42 (95%)	0.89	1	1	0.92	0.9
104	42	100	42	41/42 (98%)	0.95	1	1	0.96	0.95
106	42	92.86	39	39/39 (100%)	1	1	1	1	1
107	42	100	42	42/42 (100%)	1	1	1	1	1
109	42	97.62	41	41/41 (100%)	1	1	1	1	1
110	42	95.24	40	40/40 (100%)	1	1	1	1	1
112	42	92.86	39	39/39 (100%)	1	1	1	1	1
116	42	97.62	41	40/41 (98%)	0.95	1	1	0.96	0.95
119	42	97.62	41	41/41 (100%)	1	1	1	1	1
124	42	97.62	41	41/41 (100%)	1	1	1	1	1
127	42	100	42	38/42 (90%)	0.79	1	1	0.85	0.8
135	42	97.62	41	41/41 (100%)	1	1	1	1	1
144	42	100	42	42/42 (100%)	1	1	1	1	1
146	42	97.62	41	41/41 (100%)	1	1	1	1	1
148	42	100	42	42/42 (100%)	1	1	1	1	1
149	42	92.86	39	39/39 (100%)	1	1	1	1	1
151	42	97.62	41	41/41 (100%)	1	1	1	1	1
159	42	100	42	42/42 (100%)	1	1	1	1	1
161	42	95.24	40	40/40 (100%)	1	1	1	1	1
162	42	90.48	38	38/38 (100%)	1	1	1	1	1
168	42	95.24	40	40/40 (100%)	1	1	1	1	1
170	42	100	42	42/42 (100%)	1	1	1	1	1
191	42	100	42	42/42 (100%)	1	1	1	1	1
198	42	97.62	41	40/41 (98%)	0.95	1	1	0.96	0.95
199	42	100	42	42/42 (100%)	1	1	1	1	1
202	42	95.24	40	40/40 (100%)	1	1	1	1	1
207	42	97.62	41	41/41 (100%)	1	1	1	1	1
210	42	100	42	42/42 (100%)	1	1	1	1	1

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Lab/ Core	102	103	107	110	111	112	114	116	119	124	127	132	144	146	148	159	162	168	170	190	191	198	199	202	207	XXX	Histotype
1	N	N	N	N	N	N	N	N	P	P	N	P	N	N	N	N	N	N	N	P	N	N	N	N	N	N	EC
2	U	N	N	N	N	U	N	N	P	P	N	P	N	N	N	N	N	U	N	P	N	N	N	N	N	U	MC
3	N	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	EC
4	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	N	P	P	P	N	P	P	P	P	P	P	CCC
5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	CCC
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	HGSC
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	EC
8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
9	U	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	U	P	P	P	P	P	P	P	P	CCC
10	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	CCC
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	LGSC
12	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	LGSC
13	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
14	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	CCC
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
16	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U	P	P	CCC
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	EC
18	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	EC
20	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	LGSC
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	HGSC
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
24	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	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	U	EC
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	HGSC
27	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	CCC
28	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
29	N	N	N	N	N	N	N	N	P	P	N	P	N	N	N	N	N	N	N	P	N	N	N	N	N	N	EC
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
31	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U	U	U	P	P	CCC
32	N	N	N	U	N	U	N	U	U	U	N	N	N	U	N	N	U	N	N	N	N	N	N	U	U	U	HGSC
33	N	N	N	N	N	N	N	N	P	P	N	P	N	N	N	N	N	N	N	P	N	N	N	N	N	N	MC
34	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	HGSC
35	N	N	N	P	N	N	N	N	P	P	N	N	N	P	N	N	N	N	N	P	P	P	N	N	N	P	CCC
36	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	LGSC
37	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
38	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	CCC
39	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC
40	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	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	EC
42	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	HGSC

Key: P = Positive N = Negative U = Unsatisfactory

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Napsin Descriptive Statistics

Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
102	42	92.86	37	36/37 (97%)	0.91	1	1	0.96	0.93
103	42	100	39	38/39 (97%)	0.92	1	1	0.96	0.94
107	42	100	39	38/39 (97%)	0.92	1	1	0.96	0.94
110	42	97.62	39	39/39 (100%)	1	1	1	1	1
111	42	100	39	38/39 (97%)	0.92	1	1	0.96	0.94
112	42	95.24	39	38/39 (97%)	0.92	1	1	0.96	0.94
114	42	100	39	38/39 (97%)	0.92	1	1	0.96	0.94
116	42	97.62	39	38/39 (97%)	0.92	1	1	0.96	0.94
119	42	97.62	39	36/39 (92%)	1	0.89	0.8	1	0.83
124	42	97.62	39	36/39 (92%)	1	0.89	0.8	1	0.83
127	42	100	39	37/39 (95%)	0.83	1	1	0.93	0.87
132	42	100	39	35/39 (90%)	0.92	0.89	0.79	0.96	0.77
144	42	100	39	38/39 (97%)	0.92	1	1	0.96	0.94
146	42	97.62	39	39/39 (100%)	1	1	1	1	1
148	42	100	39	37/39 (95%)	0.83	1	1	0.93	0.87
159	42	100	39	36/39 (92%)	0.75	1	1	0.9	0.81
162	42	97.62	39	37/39 (95%)	0.83	1	1	0.93	0.87
168	42	95.24	38	37/38 (97%)	0.91	1	1	0.96	0.93
170	42	97.62	38	36/38 (95%)	0.92	0.96	0.92	0.96	0.88
190	42	100	39	35/39 (90%)	0.92	0.89	0.79	0.96	0.77
191	42	100	39	39/39 (100%)	1	1	1	1	1
198	42	97.62	38	38/38 (100%)	1	1	1	1	1
199	42	97.62	38	37/38 (97%)	0.91	1	1	0.96	0.93
202	42	88.1	35	34/35 (97%)	0.9	1	1	0.96	0.93
207	42	97.62	39	38/39 (97%)	0.92	1	1	0.96	0.94

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p53 STAINING PROTOCOLS

Lab	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	TIme for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
102	Dako 3 in 1 High pH	10/20/10	D0-7	1/100	Dako	20007332	30" RT	Dako Flex	N	Y	DAB+
103	CC1	36 MINS	BP53-11	PRE	VENTANA	E05536	32 MINS	DAB	N	Y COPPER	DAB
104	HIER	20'	PAB1801	1:1000	Calbiochem	D00119094	30'	Polymer	Yes	No	DAB
106	EDTA microwave	10 min	DO-7	1:100	biocare	81012	30 minutes	MACH4 mouse	no	no	DAB
107	cc1	24	DO-7	1:100	Dako	20007331	32 min at 37 C	Optiview DAB	N	Y	DAB
109	HIER CC1	56 MIN	Bp53-11	RTU	VENTANA	E03410	16 MIN	OPTIVIEW	NO	YES	DAB
110	PT - pH9	20 min	D0-7	1:800	DAKO	85287	30 min	Dako FLEX	N	N	DAB
111	CC1	36 MIN	Bp53-11	PREDILUTE	VENTANA	E05536	32 MIN	iVIEW	N	COPPER	DAB
112	BOND ER 2 pH 9.0	30	DO-7	RTU	LEICA	27952	15	BOND Polymer	NO	NO	DAB
114	CC1	32	DO-7	1:400	dako	54400	16	opitveiw	n	y	dab
116	CC1	60 min	D07	1/100	Cell Marque	1304307 C	32 min	ultraView DAB	N	copper	DAB
119	HIER	30 min	Bp53-11	Pre-diluted	Ventana	D06079	32 min	UltraView	N	-	DAB
124	Chaleur	60	DO-7	Pre-diluted	Ventana	E03348	32	Ultra view (Multimer)	N	N	DAB
127	automated (ultra cell	52 Min.	DO-7	Predilute	Ventana	D11087	36 Min.	Ultra View DAB	Y	N	DAB
132	Envision High pH	20 minutes	DO-7	RTU	Dako	20010412	20 minutes	Flex	None	None	DAB
133	CC1	64	DO7	predilute	Ventana	E03348	32	Optiview	n	n	dab
139	cc1 high ph	30 min	Bp53-11	ready use	Ventana	C12402	32 min	Iview DAB	no	no	iview dab
144	CC1	16	Bp53-11	neat	Ventana	E03410	16	Optiview	N	Y	DAB
147	Bond ER2 (pH9), on	20	DO-7	1:4000	DAKO	71709	15	BOND REFINE	N	N	DAB
148	cc1	64	p53 DO7	RTU	Ventana	E03348	16	Ultraview DAB	N	Y	DAB
149	PT Link high pH	20 min	D07	RTU	Dako IR616	20002492	20 min	Envision Flex	N	N	DAB
152	HIER pH 8.0 (CC1)	30	Bp53-11	Pre-diluted	Roche-Ventana	E05288	16	iVIEW	N	Copper sulfate	DAB
153	CC1	32 min	Do-7	n/d	Ventana	E03348	16 min	Opti-View Dab	N	Y	Dab
157	CC1	24 MIN.	Bp53-11	Pre-diluted	VENTANA	E05536	8 MIN.	VENTANA XT	N	N	OPTIVIEW
159	high pH	40 total	D0-7	prediluted	DAKO	20010412	30	FLEX DAKO	N	N	DAB
161	HIER	20 minutes	DO-7	RTU	DAKO	20010412	20 minutes	Envision Flex	No	No	DAB
162	CC1 ventana	48 min	DO-7	1:2000	Dako	63444	32 min	OptiView Dab	-	-	OptiView Dab
165	hier	30 min	ab-5 (do-7)	1/800	neomarkers	186p702c	32 min	ultraview dab	n	n	dab
167	CC1	30 min	318-6-11	1/25	Dako	10082824	36 min	ultra view DAB	N	Y	DAB
168	Heat Induced	48 minutes	DO-7	RTU	Dako	20010412	20 minutes	Envision Flex +	?	N	DAB
170	water bath ph:9.0	20 min	DO-7	ready to use	Dako	20010414	20 min	EnVision Flex Dako	no	no	DAB
176	CC1	30	Bp 53-11	Predilute	Ventana	D06079	40	iView DAB	y	n	
177	CC1	30m	DO-7	1 : 100	Dako	...	32m	ultraview	y	y	DAB
191	CC1	30'	D0-7	1/100	DAKO	85381	24'	ultraview	N	N	DAB
198	HIER citrate 6.2	5 min at 110deg C	DO-7	1/200	Novocastra-Leica	6002577	30min	MACH 1 polymer	Y	N	DAB
199	Bond ER-2 (High pH)	20 min	DO-7	RTU	Leica Biosystems	25342	15 min	Bond Refine	N	N	DAB
202	CITRATE BUFFER	20	26588	RTU	LEICA	26588	15	LEICA REFINE	N	N	DAB
207	HET RETREVAL-	76 min	DO7	Prediluted	Ventana	E05695	32 min	Ultraview	N	N	DAB-Ultra
209	HIER using Hihg pH	20 at 97C and 20 mins	DO-7	Pre dilute	Dako	95767	30 Mins	Envision	N	N	
210	High	25	DO-7	RTU	Dako	Unknown	30	Envision flex	Unknown	N	DAB

WT1 STAINING PROTOCOLS

Lab	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	TIme for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
102	Dako 3 in 1 High pH	10/20/10	WT49	1/20	Novocastra	6029026	30" RT	Dako Flex	No	Yes	DAB+
103	CC1	36 MINS	6F-h2	PRE	CELL MARQUE	1330804D	16 MINS	DAB	N	Y COPPER	DAB
104	HIER	20'	6F-H2	1:500	DAKO	10085591	20'	Polymer	No	No	DAB
106	EDTA microwave pressure	10 minutes	6F-H2	1:150	Dako	10087087	30 minutes	MACH4 mouse	no	no	DAB
107	cc1	56	WT49	1:10	Novocastra/Leica	6029026	60 min at 37 C	Optiview	N	Y	DAB
109	HIER CC1	32 MIN	6F-H2	RTU	CELL MARQUE	1301705D	32 MIN	OPTIVIEW-VENTANA	N	Y	DAB
110	PT - pH9	20 min	6F-H2	1:200	DAKO	10080000	30 min	Dako FLEX	N	N	DAB
112	BOND ER 2 pH 9.0	30	WT49	RTU	LEICA	28124	15	Bond Polymer Refine	NO	NO	DAB
116	CC1	64 min	6F-H2	1/100	Dako	10088310	36 min	ultraView DAB	yes	copper	DAB
119	HIER	60 min	6F-H2	Prediluted	Ventana	1330804B	32 min	UltraView	Y	-	DAB
124	Chaleur	60	6F-H2	Prediluted	Cell Marque	1330804D	32	DAB IView (biotin/steptavidin)	Y	N	DAB
127	automated (ultra cc1)	36 Min.	6F-H2	predilute	Cell Marque	1330804C	32 Min.	Ultra View DAB	N	N	DAB
132	Envision High pH	20 minutes				1218501A	20 minutes	Flex			DAB
135	EDTA BUFFER 99 C	20MIN	WT49	1:20	NOVOCASTRA	6018693	15MIN	POLYMER	N	N	DAB
144	CC1	32	6F-H2	1:25	Dako	10088310	32	Optiview	N	Y	DAB
146	Flex TRS High	20	6F-H2	RTU	Dako	10086975	30	Envision Flex	N	N	DAB
148	CC1	64	6F-H2	RTU	Ventana	1330804d	24	Ultraview DAB	N	Y	DAB
149	PT Link high pH	20 min	6F-H2	RTU	Dako	10088713	20 min	Envision Flex	Y	N	DAB
151	pH 9 EDTA	20 min	WT49	1:20	NCL/Leica	6024511	15 min	Polymer Bond Refine	N	N	DAB
159	high pH	40 total	6F-H2	pre diluted	DAKO	10081514	20	FLEX DAKO	N	N	DAB
161	HIER	20 minutes	6F-H2	RTU	DAKO	10088713	20 minutes	Envision Flex	No	No	DAB
162	CC1 ventana	48 min	6F-H2	1:400	Cell Marque	1206160B	32 min	OptiView DAB	-	-	OptiView DAB
168	HIER	48 minutes	6F-H2	RTU	Dako	10088713	20 minutes	Envision Flex +	?	Y: Mouse Linker	DAB
170	water bath ph:9.0	20 min	6F-H2	RTU	Dako	10084974	20 min	EnVision Flex Dako	yes	no	DAB
191	CC1	60'	6F-H2	1/100	DAKO	10060986	32'	ultraview	y	N	DAB
198	HIER citrate 6.2	5 min 110deg C	6F-H2	1/500	DAKO	10039600	30min	MACH 1 polymer	N	Y	DAB
199	Bond ER-2 (high pH)	30 min	WT49	RTU	Leica Biosystems	26289	15 min	Bond Refine Detection	N	N	DAB
202	CITRATE PH 9.5	40	WT49	RTU	LEICA	26289	15	LEICA REFINE SYSTEM	N	N	DAB
207	HET RETREVAL-CC1	52 min	6F-H2	Prediluted	Ventana	1424502B	32 min	ultraview	N	N	DAB Ultra
210	High	25	6F-H2	RTU	Dako	unknown	20	Envision flex+	Unknown	Mouse linker	DAB

NAPSIN-A STAINING PROTOCOLS

Lab	Ag Retrieval	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/	Ab Lot #	TIme for Ab Incubation (min)	Detection Sy	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
102	Dako 3 in 1 High pH	10/20/10	TMU-Ad 02	1/50	Biocare	11614	30" RT	Dako Flex	N	Y	DAB+
103	CC1	20	IP64	1/100	LEICA	6026035	32 RT	DAB	N	Y COPPER	DAB
107	cc1	32	IP64	1:200	Novocastra/ Leica	6026035	32	Optiview	N	Y	DAB
110	PT - pH9	20 min	TMU-Ad02	1:100	Biocare	11614	30 min	Dako FLEX	Y - 15 MIN MULTIMER	N	DAB
111	CC1	36 MIN	IP64	1/100	Leica Novacastra	6026035	32 min	ULTRAVIEW	N	COPPER	DAB
112	BOND ER 2 pH 9.0	25	IP64	1:1000	LEICA	6011320	15	Bond Polymer Refine	NO	NO	DAB
114	CC1	32	poly	1:200	cell marque	1035702B	16	optiview	n	y	DAB
116	CC1	36 min	TMU-Ad02	1/50	Biocare Medical	80113	44 min	ultraView DAB	No	copper	DAB
119	HIER	30 min	polyclonal	Predilute	Ventana	1323407C	32 min	UltraView	N	-	DAB
124	Chaleur	30	polyclonal	Predilute	cell Marque	1323407E	32	DAB IView (Biotin/stept avidin)	N	N	DAB
127	automated (ultra cc1)	36 Min.	Napsin A polyclonal	predilute	Cell Marque	1323407B	12 Min.	Ultra View DAB	N	N	DAB
135	ER2	20MIN	WT49	1:20	NOVOCASTRA	6018693	15MIN	POLYMER	N	N	DAB
144	CC1	32	IP64	1:200	Novocastra	6026035	32	Optiview	N	Y	DAB
146	Flex TRS High	20	TMU-Ad 02	1/100	BioCare	11013	20	Envision Flex	N	N	DAB
148	CC1	36	Napsin Polyclonal	RTU	Ventana	1404205b	24	Ultraview DAB	N	Y	DAB
159	low pH	40 total	TMU-Ad 02	1/100	biocare	102014	30	DAKO Flex	No	No	DAB
162	CC1 ventana	48 min	IP64	1:200	Trichem	6017974	32 min	OptiView DAB	-	-	OptiView DAB
168	HIER	48 minutes	TMU-ad02	1/200	Biocare	80113	30 minutes	Envision Flex +	?	Y: Mouse Linker	DAB
170	water bath ph:9.0	20 min	MRQ-60	1/100	Dako	1233303c	20 min	EnVision Flex Dako	no	no	DAB
191	CC1	30'	TMU-Ad02	1/400	IBL	6026035	32'	ultraview	N	N	DAB
198	HIER citrate 6.2	5min 110deg C	TMU-Ad02	1/100	Biocare Med-InterMedico	40512	60min	MACH 1 polymer	N	Y	DAB
199	Bond ER-1 (low pH)	30 min	IP64	1:500	Leica Biosystems	6011320	15 min	Bond Refine Detection	N	N	DAB
202	none	none	IP64	1/100	NOVOCASTRA/LEICA	6017974	15	LEICA REFINE SYSTEM	N	N	DAB
207	HET RETREVAL-CC1	64 min	TMU-Ad 02	1/100	Biocare	30112	32	Ultra view	N	N	DAB Ultraview