



building towards

canadian **I**mmunohistochemistry Quality control

CIQC

Results of Assessment Meeting for CIQC Run 59, Breast Biomarkers

Performed at Vancouver General Hospital, on Monday June 20, 2016

Assessors: Blake Gilks, Jennifer Won, John Garratt

Background

This run consisted of 40 cores, stained with the breast biomarker panel (ER, PR and HER2). Breast biomarker results, overall, remain excellent. General comments about each of the three markers follow, and then comments for individual laboratories based on review of their ER slides. We have not commented specifically on a lab-by-lab basis for PR and HER2, for the reasons noted below.

ER

ER testing is a foundational aspect of modern anatomic pathology practice, and excellent staining results are to be expected. As is usual with ER testing in current practice, most tumors are either completely negative or show strong diffuse staining (Allred 8/8). For proficiency testing purposes, the most informative cores are those with weaker staining e.g. cores 13, 21 and 30. A lab was considered to have optimal staining if results were completely concordant with reference or at most one of the weakly staining tumor cores was negative. If two or three of the weakly staining cores were negative, staining was considered adequate (five labs). If there were false positive results or more than 3 false negative results, staining was considered suboptimal (two labs). Should labs with adequate or suboptimal staining results wish to repeat testing or adjust their protocols, please feel free to contact us to request additional unstained slides for testing.

We have drawn your attention to an emerging trend over the past few years, i.e. that weak ER positive cases (i.e. Allred scores 3-5) are not the same as the strongly positive cases. A recent publication makes the point that such weakly positive tumors are more similar to triple negative breast cancers, with respect to their underlying risk of BRCA1 mutation, adding to the body of evidence that weak ER positivity in the era of highly sensitive testing has different implications than strong positivity, and some tumors that were formerly triple-negative, are now weak ER positive (Sanford RA, Song J, Gutierrez-Barrera AM et al. High Incidence of Germline BRCA Mutation in Patients with ER Low-Positive/PR Low-Positive/HER-2 neu Negative Tumors. *Cancer* 2015;121:3422-7). As noted previously, don't think that any action is required with respect to how we are performing ER testing, beyond being aware of this phenomenon.

ER - Assessors' Comments

101	optimal	
102	optimal	
106	optimal	
109	optimal	
111	optimal	
112	optimal	
114	optimal	
115	No slides available for review	
116	optimal	
119	optimal	
120	optimal	
122	optimal	
123	optimal	
124	adequate	Cores 21 and 30 were negative, Slightly weak staining
125	optimal	
126	optimal	
127	optimal	
128	optimal	
129	optimal	
132	optimal	
133	optimal	
134	optimal	
138	adequate	Cores 21 and 30 were negative, Slightly weak staining
139	optimal	
141	optimal	
143	optimal	
144	optimal	
145	optimal	
146	optimal	
147	optimal	
148	optimal	
149	optimal	
150	optimal	
151	optimal	
152	optimal	
153	optimal	

155	optimal	
157	optimal	
159	No slides available for review	
160	optimal	
161	optimal	
162	optimal	
164	optimal	
165	optimal	
167	No slides available for review	
168	adequate	Cores 21, 23 and 30 were negative. Strong nuclear counterstain may have contributed to false negative results
170	optimal	
173	optimal	
175	optimal	
177	adequate	Cores 21 and 30 were negative. Slightly weak staining
178	optimal	
180	optimal	
183	optimal	
186	optimal	
187	optimal	
188	suboptimal	False positive results for cores 12, 16, 37 and 40
189	optimal	
190	optimal	
192	optimal	
194	optimal	
196	optimal	
198	suboptimal	Cores 8, 21, 23 and 30 all negative. Weak staining
199	optimal	
202	optimal	
207	optimal	
209	optimal	
215	optimal	
216	optimal	
217	optimal	
221	adequate	Cores 21 and 30 negative. Intense nuclear counterstain may have contributed to false negative results

HER2

HER2 immunostaining remains excellent. There were no false positive results! Cores 1, 2, 4, 5, 8, 11, 12, 26, 30, 31, and 36 featured HER2 amplification and protein overexpression and of more than 400 results for these cases, there were only 2 false negative results (two labs had 1+ staining for core 11), amongst those labs that returned their slides for review.

Core 23 is worth commenting on. Although there was amplification of HER2 by FISH, none of the participating labs had 3+ staining of this tumor. It is impossible to know the significance of HER2 amplification in breast cancers with relatively low level (or absent in some cases) protein expression. We do not believe that it is appropriate to adjust protocols in an attempt to achieve 2 or 3+ staining in cases such as this, where the clinical significance of amplification is not known. There will remain cases with amplification of HER2 and no detectable protein expression, where the DNA copy number changes reflect more widespread somatic copy number alterations and HER2 amplification, per se, is not a driver of tumor growth.

PR

Quality assurance/external proficiency testing for PR remains problematic, as there is no accepted “gold standard” for comparison. Using the consensus of results from labs across Canada remains the CIQC approach, and results on this basis were very good, with the exception of lab 146, where there were significantly more PR positive cases than were seen in other labs, including cases that were ER negative (e.g. cores 12 and 16) and they should investigate their staining.

Descriptive Statistics			ER						
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	37	91.89	34	33/34 (97%)	0.96	1	1	0.91	0.93
102	37	97.3	36	36/36 (100%)	1	1	1	1	1
106	37	100	37	37/37 (100%)	1	1	1	1	1
109	37	94.59	35	35/35 (100%)	1	1	1	1	1
111	37	100	37	37/37 (100%)	1	1	1	1	1
112	37	100	37	37/37 (100%)	1	1	1	1	1
114	37	100	37	37/37 (100%)	1	1	1	1	1
115	37	100	37	35/37 (95%)	0.93	1	1	0.83	0.87
116	37	100	37	37/37 (100%)	1	1	1	1	1
119	37	100	37	37/37 (100%)	1	1	1	1	1
120	37	100	37	37/37 (100%)	1	1	1	1	1
122	37	100	37	37/37 (100%)	1	1	1	1	1
123	37	100	37	37/37 (100%)	1	1	1	1	1
124	37	100	37	35/37 (95%)	0.93	1	1	0.83	0.87
125	37	100	37	37/37 (100%)	1	1	1	1	1
126	37	100	37	36/37 (97%)	0.96	1	1	0.91	0.93
127	37	100	37	37/37 (100%)	1	1	1	1	1
128	37	100	37	37/37 (100%)	1	1	1	1	1
129	37	100	37	37/37 (100%)	1	1	1	1	1
132	37	97.3	36	36/36 (100%)	1	1	1	1	1
133	37	100	37	37/37 (100%)	1	1	1	1	1
134	37	100	37	37/37 (100%)	1	1	1	1	1
138	37	100	37	35/37 (95%)	0.93	1	1	0.83	0.87
139	37	100	37	37/37 (100%)	1	1	1	1	1
141	37	89.19	33	33/33 (100%)	1	1	1	1	1
143	37	83.78	31	31/31 (100%)	1	1	1	1	1
144	37	94.59	35	35/35 (100%)	1	1	1	1	1
145	37	78.38	29	29/29 (100%)	1	1	1	1	1
146	37	86.49	32	32/32 (100%)	1	1	1	1	1
147	37	94.59	35	34/35 (97%)	0.96	1	1	0.9	0.93
148	37	89.19	33	33/33 (100%)	1	1	1	1	1
149	37	86.49	32	32/32 (100%)	1	1	1	1	1
150	37	89.19	33	33/33 (100%)	1	1	1	1	1
151	37	91.89	34	34/34 (100%)	1	1	1	1	1
152	37	91.89	34	33/34 (97%)	0.96	1	1	0.91	0.93
153	37	91.89	34	33/34 (97%)	1	0.9	0.96	1	0.93
155	37	86.49	32	32/32 (100%)	1	1	1	1	1
157	37	91.89	34	33/34 (97%)	1	0.9	0.96	1	0.93
159	37	86.49	32	31/32 (97%)	0.96	1	1	0.89	0.92
160	37	97.3	36	36/36 (100%)	1	1	1	1	1
161	37	91.89	34	34/34 (100%)	1	1	1	1	1
162	37	97.3	36	36/36 (100%)	1	1	1	1	1
164	37	94.59	35	35/35 (100%)	1	1	1	1	1
165	37	94.59	35	35/35 (100%)	1	1	1	1	1
167	37	94.59	35	34/35 (97%)	0.96	1	1	0.9	0.93
168	37	89.19	33	30/33 (91%)	0.88	1	1	0.75	0.79
170	37	94.59	35	35/35 (100%)	1	1	1	1	1
173	37	100	37	37/37 (100%)	1	1	1	1	1
175	37	97.3	36	35/36 (97%)	0.96	1	1	0.9	0.93
177	37	97.3	36	34/36 (94%)	0.93	1	1	0.82	0.86
178	37	72.97	27	27/27 (100%)	1	1	1	1	1
180	37	86.49	32	32/32 (100%)	1	1	1	1	1
183	37	86.49	32	31/32 (97%)	0.95	1	1	0.91	0.93
186	37	86.49	32	32/32 (100%)	1	1	1	1	1
187	37	81.08	30	29/30 (97%)	0.95	1	1	0.89	0.92
188	37	75.68	28	24/28 (86%)	1	0.43	0.84	1	0.53
189	37	86.49	32	31/32 (97%)	0.95	1	1	0.91	0.93
190	37	75.68	28	27/28 (96%)	0.95	1	1	0.89	0.92
192	37	97.3	36	36/36 (100%)	1	1	1	1	1
194	37	94.59	35	34/35 (97%)	0.96	1	1	0.9	0.93
196	37	100	37	37/37 (100%)	1	1	1	1	1
198	37	89.19	33	29/33 (88%)	0.83	1	1	0.69	0.73
199	37	100	37	37/37 (100%)	1	1	1	1	1
202	37	100	37	36/37 (97%)	0.96	1	1	0.91	0.93
207	37	100	37	37/37 (100%)	1	1	1	1	1
209	37	75.68	28	28/28 (100%)	1	1	1	1	1
215	37	86.49	32	32/32 (100%)	1	1	1	1	1
216	37	83.78	31	31/31 (100%)	1	1	1	1	1
217	37	83.78	31	31/31 (100%)	1	1	1	1	1
221	37	86.49	32	30/32 (94%)	0.91	1	1	0.82	0.86

Descriptive Statistics			PR						
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	36	91.67	33	31/33 (94%)	1	0.9	0.87	1	0.88
102	36	97.22	35	35/35 (100%)	1	1	1	1	1
106	36	100	36	36/36 (100%)	1	1	1	1	1
109	36	100	36	35/36 (97%)	1	0.95	0.94	1	0.94
111	36	97.22	35	35/35 (100%)	1	1	1	1	1
112	36	97.22	35	35/35 (100%)	1	1	1	1	1
114	36	97.22	35	35/35 (100%)	1	1	1	1	1
115	36	100	36	36/36 (100%)	1	1	1	1	1
116	36	100	36	32/36 (89%)	0.88	0.9	0.88	0.9	0.77
119	36	100	36	35/36 (97%)	1	0.95	0.94	1	0.94
120	36	100	36	35/36 (97%)	1	0.95	0.94	1	0.94
122	36	100	36	36/36 (100%)	1	1	1	1	1
123	36	91.67	33	33/33 (100%)	1	1	1	1	1
124	36	100	36	32/36 (89%)	1	0.8	0.8	1	0.78
125	36	100	36	35/36 (97%)	0.94	1	1	0.95	0.94
126	36	100	36	36/36 (100%)	1	1	1	1	1
127	36	100	36	34/36 (94%)	1	0.9	0.89	1	0.89
128	36	100	36	31/36 (86%)	1	0.75	0.76	1	0.73
129	36	100	36	36/36 (100%)	1	1	1	1	1
132	36	97.22	35	33/35 (94%)	1	0.9	0.88	1	0.89
133	36	100	36	36/36 (100%)	1	1	1	1	1
134	36	100	36	34/36 (94%)	1	0.9	0.89	1	0.89
138	36	91.67	33	33/33 (100%)	1	1	1	1	1
139	36	100	36	35/36 (97%)	1	0.95	0.94	1	0.94
141	36	77.78	28	28/28 (100%)	1	1	1	1	1
143	36	97.22	35	33/35 (94%)	1	0.89	0.89	1	0.89
145	36	91.67	33	33/33 (100%)	1	1	1	1	1
146	36	88.89	32	18/32 (56%)	1	0.22	0.5	1	0.2
147	36	91.67	33	33/33 (100%)	1	1	1	1	1
149	36	61.11	22	22/22 (100%)	1	1	1	1	1
150	36	88.89	32	23/32 (72%)	0.93	0.56	0.62	0.91	0.46
151	36	91.67	33	32/33 (97%)	1	0.95	0.93	1	0.94
152	36	94.44	34	34/34 (100%)	1	1	1	1	1
153	36	88.89	32	29/32 (91%)	1	0.83	0.82	1	0.81
155	36	94.44	34	30/34 (88%)	1	0.79	0.79	1	0.77
157	36	97.22	35	28/35 (80%)	1	0.65	0.68	1	0.61
159	36	97.22	35	30/35 (86%)	0.88	0.84	0.82	0.89	0.71
160	36	97.22	35	31/35 (89%)	1	0.8	0.79	1	0.77
161	36	91.67	33	33/33 (100%)	1	1	1	1	1
162	36	94.44	34	30/34 (88%)	0.75	1	1	0.82	0.76
164	36	91.67	33	32/33 (97%)	1	0.94	0.94	1	0.94
165	36	94.44	34	26/34 (76%)	0.94	0.61	0.68	0.92	0.54
167	36	91.67	33	33/33 (100%)	1	1	1	1	1
168	36	94.44	34	34/34 (100%)	1	1	1	1	1
170	36	86.11	31	31/31 (100%)	1	1	1	1	1
173	36	100	36	35/36 (97%)	1	0.95	0.94	1	0.94
175	36	97.22	35	30/35 (86%)	1	0.75	0.75	1	0.72
177	36	97.22	35	34/35 (97%)	0.93	1	1	0.95	0.94
178	36	88.89	32	29/32 (91%)	0.87	0.94	0.93	0.89	0.81
183	36	88.89	32	27/32 (84%)	1	0.74	0.72	1	0.69
186	36	94.44	34	31/34 (91%)	0.93	0.89	0.88	0.94	0.82
187	36	80.56	29	28/29 (97%)	1	0.94	0.93	1	0.93
188	36	88.89	32	29/32 (91%)	0.93	0.89	0.87	0.94	0.81
189	36	88.89	32	30/32 (94%)	0.93	0.94	0.93	0.94	0.87
190	36	94.44	34	33/34 (97%)	0.93	1	1	0.95	0.94
192	36	97.22	35	33/35 (94%)	0.93	0.95	0.93	0.95	0.88
194	36	94.44	34	34/34 (100%)	1	1	1	1	1
196	36	97.22	35	34/35 (97%)	0.93	1	1	0.95	0.94
198	36	88.89	32	31/32 (97%)	1	0.94	0.94	1	0.94
199	36	100	36	36/36 (100%)	1	1	1	1	1
202	36	91.67	33	33/33 (100%)	1	1	1	1	1
207	36	100	36	34/36 (94%)	0.94	0.95	0.94	0.95	0.89
209	36	88.89	32	29/32 (91%)	0.87	0.94	0.93	0.89	0.81
215	36	88.89	32	27/32 (84%)	1	0.72	0.74	1	0.69
216	36	86.11	31	30/31 (97%)	1	0.94	0.93	1	0.94
217	36	86.11	31	25/31 (81%)	1	0.67	0.68	1	0.63
221	36	91.67	33	31/33 (94%)	1	0.9	0.87	1	0.88

Descriptive Statistics			HER2						
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	35	94.29	32	31/32 (97%)	0.92	1	1	0.95	0.93
102	35	94.29	33	33/33 (100%)	1	1	1	1	1
106	35	100	34	34/34 (100%)	1	1	1	1	1
109	35	97.14	34	34/34 (100%)	1	1	1	1	1
111	35	97.14	33	33/33 (100%)	1	1	1	1	1
112	35	100	34	34/34 (100%)	1	1	1	1	1
114	35	97.14	33	33/33 (100%)	1	1	1	1	1
115	35	100	34	34/34 (100%)	1	1	1	1	1
116	35	100	34	34/34 (100%)	1	1	1	1	1
119	35	100	34	34/34 (100%)	1	1	1	1	1
120	35	100	34	34/34 (100%)	1	1	1	1	1
123	35	97.14	33	33/33 (100%)	1	1	1	1	1
124	35	100	34	32/34 (94%)	0.85	1	1	0.91	0.87
125	35	100	34	34/34 (100%)	1	1	1	1	1
126	35	100	34	34/34 (100%)	1	1	1	1	1
127	35	100	34	34/34 (100%)	1	1	1	1	1
129	35	100	34	34/34 (100%)	1	1	1	1	1
133	35	100	34	34/34 (100%)	1	1	1	1	1
138	35	91.43	31	31/31 (100%)	1	1	1	1	1
139	35	100	34	34/34 (100%)	1	1	1	1	1
145	35	82.86	29	29/29 (100%)	1	1	1	1	1
147	35	85.71	30	30/30 (100%)	1	1	1	1	1
149	35	91.43	32	32/32 (100%)	1	1	1	1	1
150	35	94.29	32	31/32 (97%)	0.92	1	1	0.95	0.93
151	35	94.29	33	32/33 (97%)	0.92	1	1	0.95	0.94
152	35	91.43	31	31/31 (100%)	1	1	1	1	1
153	35	94.29	32	32/32 (100%)	1	1	1	1	1
155	35	91.43	31	31/31 (100%)	1	1	1	1	1
157	35	94.29	32	32/32 (100%)	1	1	1	1	1
160	35	94.29	32	32/32 (100%)	1	1	1	1	1
161	35	91.43	31	30/31 (97%)	0.92	1	1	0.95	0.93
162	35	97.14	33	33/33 (100%)	1	1	1	1	1
164	35	94.29	32	32/32 (100%)	1	1	1	1	1
167	35	94.29	32	32/32 (100%)	1	1	1	1	1
168	35	94.29	32	32/32 (100%)	1	1	1	1	1
170	35	100	34	34/34 (100%)	1	1	1	1	1
175	35	97.14	33	33/33 (100%)	1	1	1	1	1
186	35	94.29	33	32/33 (97%)	0.92	1	1	0.95	0.94
187	35	82.86	28	28/28 (100%)	1	1	1	1	1
188	35	82.86	29	27/29 (93%)	0.82	1	1	0.9	0.85
189	35	94.29	33	33/33 (100%)	1	1	1	1	1
190	35	97.14	33	33/33 (100%)	1	1	1	1	1
194	35	97.14	33	33/33 (100%)	1	1	1	1	1
198	35	100	34	34/34 (100%)	1	1	1	1	1
199	35	100	34	34/34 (100%)	1	1	1	1	1
202	35	97.14	33	33/33 (100%)	1	1	1	1	1
207	35	100	34	34/34 (100%)	1	1	1	1	1
215	35	94.29	32	32/32 (100%)	1	1	1	1	1
216	35	94.29	32	32/32 (100%)	1	1	1	1	1
217	35	94.29	32	30/32 (94%)	0.85	1	1	0.9	0.87
221	35	94.29	32	31/32 (97%)	0.92	1	1	0.95	0.93

ER

Labs/Fields	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	Labs/Fields	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 minutes	SP1	1:50	Thermo	9101S1501E	32 minutes	OptiView	N	Y	DAB	153	HER CC1	32	SP1	Predilute	Ventana	F07779	8	optiview	N	Y	DAB
102	DAKO PT - High pH TRS	10/20/10	EP1	1:40	DAKO	10099710	30' RT	DAKO FLEX	NO	CUSO4	DAB+	155	CC1	30	SP1	Predilute	Ventana	F08061	40	Ultraview dab	non	yes	dab
106	CC1	64	SP1	predilute	Roche	F08061	32	Optiview	no	yes	DAB	157	CC1	24 MIN	SP-1	PRE DILUTED	VENTANA	F08061	24 MIN	OPTVIEW/BENCHMARK XT	Y	Y	DAB
109	HER HIGH pH (CC1)	64 MIN	SP1	rtu	ROCHE/VENTANA	F04055	32 MIN	ULTRAVIEW	NO	YES	DAB	159	Flex TRS High	40 min. total	EP1	none prediluted	Dako	10108187	20 min.	Flex	N	N	Flex Dab
111	CC1	36 MIN	SP1	PREDILUTE	VENTANA	F02583	32 MIN	ULTRAVIEW	N	Y	COPPER	160	EDTA pH8	36	SP1	No	Ventana	?	8	Ultraview	N	N	DAB
112	Bond ER2 pH 9.0	20 minutes	SP1	1:150	ThermoFisher	QF2032871	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB	161	HER-High EDTA TRS tampon	20 Minutes	EP1	RTU	DAKO	10108187	20 Minutes	Envision Flex	No	No	DAB
114	CC1	32	SP1	1:50	Thermo Fisher	9101S1501E	16	Optiview Envision Flex	N	Y	DAB	162	CC1 ventana	48 min	SP1	1:100	Thermo Scientific	9101s1501G	32 min	Optiview DAB, Ventana	N	N	Optiview DAB Ventana
115	HER	30 mins	EP1	RTU	Dako	10110172	30 mins	ULTRAVIEW DAB	N	N	DAB	164	ultraCC1	36	SP1	predilute	Ventana	F08061	8	Ultraview	n	n	DAB
116	CC1	64 min	sp1	RTU	VENTANA	F07779	32 MIN	ULTRAVIEW DAB	N	.	DAB	165	hier	30 min	sp1	nil	ventana	108061	28 min	ultraview	n	n	dab
119	HER/CC1	30 min	SP1	pre-diluted	Ventana	F08061	24 min	Ultraview	nil	nil	DAB	167	CC1	30 min	SP1	pre-diluted	Roche	F05106	8 min	Ultraview DAB kit	N	Y (copper)	DAB
120	Waterbath	20	alpha EP1	Pre-dilute	Dako	10100789	20	Autostainer Ink48 - Envision Flex	N	N	DAB	168	HER	48	SP1	1/100	CellMarque	1422401H	30	Envision Flex +	N	Y	DAB
122	HER PH 9	20	SP1	N/A	VENTANA	E10753	15	POLYMER REFINE	N	N	DAB	170	HER	20 min	Ep1	ready to use	Dako	106139	20 min	Envision Flex	N	N	DAB
123	CC1	36	SP1	PREDILUTE	VENTANA/ROCHE	F07779	32	ULTRAVIEW	N	Y	DAB	173	CC1 EDTA	30	sp1	pre-diluted	Ventana	F06774	16	Ultraview polymer	N	Y	DAB
124	CC1	60 min	SP1	1/100	Cell marque	1422401B	32	Ultra view	n	n	DAB	175	HER	36	SP1	pre-dilute	Roche	F08061	32	Ultraview	N	Y	DAB
125	Omnis TRIS H	30	SP1	predilute	ROCHE	f07779	20	OMNIS ENVISION FLEX	Y	N	DAB	177	CCI	30 min	6F11	1:25	Novocastra	-	32 min	Ultraview	y	y	DAB
126	Steam Citrate, ph 6.01	45 minutes	SP1	1:200	Thermo Scientific (NeoMarkers)	9101S1501E	30 minutes	Envision + Rabbit	N	N	DAB +	178	HER	32	SP1	none	Ventana	n	16	Ultraview	n	n	DAB
127	automated (CC1 on benchmark)	36 minutes	SP1	predilute	Ventana	F02583	32 minutes	ultraview detection kit	N	N	DAB	180	CC1	32	SP1	RTU	VENTANA	F04320	16	OPTVIEW	N	N	DAB
128	CC1	64 min	SP1	Pre-dilute	Ventana	F04055	16 min	Ultraview	No	Yes	DAB	183	Ultra CC1	36	SP1	predilute	Roche	F04055	32	Ultraview	n	n	DAB
129	ER2 - high pH retrieval	20	SP1	1:100	Thermo Scientific	9101S1501B	15	Bond Refine Detection Kit	No	No	DAB	186	HER	20 MIN	SP1	1:50	THERMOSCI ENTIFIC	9101S101E	15 MIN	DAB BOND POLYMER	N	N	DAB
132	flex high pH	20	EP1	RTU	DAKO	10106139	20	ENVISION FLEX	NONE	NONE	DAB	187	CC1	16	SP1	Predilute	Roche	F08061	8	Optiview	None	None	DAB
133	hier	36	SP1	predilute	Roche	F00389	32	Ultraview	n	n	DAB+	188	HER ER 1 BOND	20	6F11	BOND RTU	LEICA	42760	15	BOND POLYMER REFINE KIT	N	N	DAB
134	CC1	30	SP1	RTU	ROCHE	F00389	8	ULTRAVIEW	N	N	DAB	189	CC1	64	SP1	pre-dilute	Ventana	unknown	16	ultraVIEW	N	N	ultraView DAB
138	HER - EDTA	20	EP1	RTU	Dako	10108187	20	Polymer - Envision FLEX	N	N	DAB	190	CC1	32	SP1	pre-diluted	VENTANA	F08061	32	I/VIEW	N	N	DAB
139	HER	30 min.	SP1	ready use	Ventana	f08061	32 min.	view	no	no	dab	192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	F04055	16 minutes	VentanaUltraview DAB	N	Y (copper)	DAB
141	CC1	30 min	SP1	RTU	Roche	F00389	8 min	ultraView	N	N	DAB	194	CC1	30	SP1	PREDILUTE	VENTANA	F07779	12 MINS.	I/VIEW KIT(AVIDIN BIOTIN)	Y	Y	DAB
143	HER, automated online system	> 30 but <45 min	SP1	no	Conf/irm Ventana/Roche	F06378	16 min	Optiview detection Kit	no	yes	DAB	196			SP1		VENTANA	F08061	8.0 MIN	DAB			
144	CC1	24 min	SP1	1:50	Leica (ThermoScientific)	9101S1406K	16 min	Optiview	No	Yes, Copper	DAB	198	Citrate pH 6.2 pressure cooker in microwave	5 min	6F11	1/150	Leica	6027943	30 min	MACH 1 polymer	Y	Y	DAB
145	CC1	32	SP1	1/100	CELLMARQUE	1316403E	16	XT OPTVIEW Inc v4	no	no	DAB	199	HER	30	6F11	RTU	Leica	40820	15	Refine	N	N	DAB
146	FLEX TRS High	20	EP1	RTU	Dako	10100789	20	EnVision FLEX	N	N	DAB	202	citrate ph 9.5	20 min	6f11	1/300	vector	3027092	15 min	Refine Detection kit Leica	no	no	DAB
147	HER, pH8	20	SP1	1:150	ThermoScientific	9101S1501J	15	Polymer (Leica Refine)	N	N	DAB	207	CC1-ON LINE	36MN	SP1	PREDILUTE	VENTANA	F07779	16 MIN	ULTRAVIEW	N	N	DAB
148	VENTANA CC1	36min	SP1	RTU	VENTANA	F08061	12	ULTRAVIEW DAB	N	N	DAB	209	HER	20mins at 97C and then 20mins cooling down to 85C	EP1	Pre dilute	Dako	10106139	20mins	Envision plus	N	N	DAB
149	PT Link high pH	20	EP1	RTU	Dako	10106139	20	EnVision Flex	No	No	DAB	215	HER	64	SP1	pre-dilute	ROCHE	F08061	32	Ultraview	n	n	DAB
150	cc1	30min	SP1	n/a	Ventana	F04055	16min	ultraview	n	y	DAB	216	HER	30	SP1	PRE-DILUTE	VENTANA/ROCHE	F08061	24	AVDINBIOTIN (I/VIEW DAB)	N	Y	DAB
151	Bond HER 2	20 min	SP1	1:50	Thermo Roche	9101S1501J	15 min	Bond Refined Polymer	N	N	DAB	217	CC1	64	SP1	pre-dilute	Roche Ventana	E10753	20	Optiview	No	No	DAB
152	HER	60	SP1	Pre-diluted	Ventana	F05106	16	I/VIEW	N	Y	DAB	221	Buffer Citrate pH8	20 Minutes	SP1	1:100	Cell Marque	14224016	30 Minutes	Rabbit EnVision	N	N	DAB

HER2

Laboratories/Fields	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 minutes	SP3	1:200	Thermo	9103S1306A	32 minutes	OptiView	N	Y	DAB
102	DAKO PT - High pH TRS	10/20/10	SP3	1:200	LABVISION	9103S1507A	30' RT	DAKO FLEX	NO	CUSO4	DAB+
106	CC1	36	4B5	predilute	Roche	F08704	16	Ultraview	no	yes	DAB
109	HER HIGH pH (CC1)	36 MIN	4B5	RTU	ROCHE/VENTANA	F07132	16 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	36 MIN	4B5	PREDILUTE	VENTANA	F06908	32 MIN	ULTRAVIEW	N	Y	COPPER
112	BOND ER2 pH 9.0	20 minutes	4B5	1:3 ration of the RTU	Ventana	E09042	15 minutes @ RT	BND POLYMER REFINE	NO	NO	DAB
114	CC1	32	SP3	1/200	Thermo Fisher	9103S1509D	32	Optiview	N	Y	DAB
115	HER	40 mins	Hercept test	RTU	Dako	20029677	30 mins	Hercept test	N	N	DAB
116	CC1	40 MIN	SP3	1/100	THERMOSCIENTIFIC	1507 F	36 MIN	OPTIVIEW DAB	N	.	DAB
119	HER/CC1	30 min	4B5	pre-diluted	Ventana	F08047	16 min	Ultraview	nil	nil	DAB
120	water bath	40	HER2 protein	pre-dilute	Dako	20028925	30 min	Autostainer Link 48 - Herceptest	N	N	DAB
123	CC1	36	4B5	PREDILUTE	VENTANA/ROCHE	F06908	24	ULTRAVIEW	N	Y	DAB
124	CC1	8 min	4B5	Predilute	Ventana	F08047	20 min	Ultra view	n	n	DAB
125	VENTANA CC1	36	4B5	predilute	ROCHE	F08704	16	VENTANA ULTRAVIEW	N	Y	DAB
126	Steam TRIS, pH 10.01 automated (CC1 on benchmark)	55 minutes	SP3	1:100	ThermoFisher/Lab Vision	9103S15098B	30 minutes	Envision + Rabbit	N	N	DAB +
127	Ultra	36 minutes	4B5	predilute	Ventana	F09704	24 minutes	ultraview detection kit	N	N	DAB
129	ER2 - high pH retrieval	20	SP3	1:100	Thermo Scientific	RA2136461	15	Bond Refine Detection Kit	N	N	DAB
133	hier	36	4B5	predilute	Roche	F06908	24	Ultraview	n	n	DAB+
138	HER - citrate	40	HercepTest	RTU	Dako	20029677	30	Polymer - HercepTest Kit	N	N	DAB
139	HER automated	30 min.	4B5	ready use	Ventana	f06908	48	Ultraview	no	no	dab
147	HER, pH9	20	SP3	1:50	ThermoScientific	RA2131961	15	Polymer (Leica Refine)	N	N	DAB
149	PT Link low pH	20	A0485	1:500	Dako	20028925	20	EnVision Flex	Yes	No	DAB
150	cc1	30min	SP3	1/100	NeoMarkers	9103S1407E	32min	ultraview	n	y	DAB
151	Bond HER 1	20 min	Polyclonal	1:500	DAKO	20002512A	15min	Bond Refine Polymer	N	N	DAB
152	HER	60	4B5	Pre-diluted	Roche Ventana	F08704	32	DAB	N	Y	DAB
153	HER CC1	32	4B5	Predilute	VENTANA	F07132	32	OPTIVIEW	N	Y	DAB
155	CC1	30	4B5	Predilute	Ventana	F07132	32	ultraview	n	y	chromogen
157	CC1	24 MIN.	4B 5	PRE DILUTED	VENTANA	F07132	24 MIN.	OPTIVIEW BENCHMARK XT	Y	Y	DAB
160	EDTA pH8	36	4B5	No	Ventana	?	12	Ultraview	N	N	DAB
161	Herceptest epitope	40 Mutes	Rabbit anti-human HER2 protein	RTU	DAKO	20029677	30 Minutes	Herceptest visualization reagent	No	No	Herceptest DAB chromogen
162	Ventana	32 min	4B5	RTU	Roche	163442	20 min	UltraView DAB	N	N	UltraView DAB
164	ultraCC1	36	4B5	predilute	Ventana	F06908	12	Ultraview	n	n	DAB
167	CC1	30 min	4B5	pre-diluted	Roche	F06908	8 min	ultraview dabkit	N	Y (copper)	DAB
168	Cell Conditioning	30	4B5	RTU	Roche	F08704	16	Ultraview DAB	N	Y	DAB
170	HER	40 min	Her 2 protein	ready to use	Dako	20028925	30 min	Herceptest	N	N	DAB
175	HER	36	4B5	pre-dilute	Roche	F08704	16	Ultraview	N	Y	DAB
186	HER	20 MIN	HER2	1:400	DAKO	20002512	15 MIN	DAB BOND POLYMER	Y	N	DAB
187	CC1	16	4B5	Predilute	Roche	F09740	24	Optiview	None	None	DAB
188	HER ER 1 BOND	25	CB11	BOND RTU	LEICA	40801	30	BOND Oracle HER2 IHC System	N	N	DAB
189	CC1	32	4B5	pre-dilute	Ventana	unknown	16	ultraView	N	N	ultraView DAB
190	CC1	32	SP3	1:50	THERMOFISHER	9103S1507F	40	VIEW	Y	N	DAB
194	CC1	30	4B5	PREDILUTE	VENTANA	F09704	12 MINS	VIEW KIT (AVIDIN BIOTIN)	N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Ventana/Roche	F08047	20 min	Ultraview	N	Y	DAB
199	HER	30	CB11	RTU	Lecia	41313	30	Oracle	N	N	DAB
202	Herceptest Epitope Retrieval ph 6.0	40 min	Her2	RTU DAKO Herceptest	DAKO	20029677	30 min	Herceptest kit DAKO	no	no	DAB
207	CC1-ON LINE	36 MIN	4B5	PREDILUTE	VENTANA	F09704	16 MIN	ULTRAVIEW	N	N	DAB
215	HER	36	4B5	pre-dilute	ROCHE	F08704	12	Ultraview	n	n	DAB
216	HER	30	4B5	PRE-DILUTE	VENTANA/ROCHE	F09704	32	AVIDINBIOTIN (VIEW DAB)	N	Y	DAB
217	CC1	32	4B5	pre-dilute	Roche Ventana	E07785	20	Optiview	No	No	DAB
221	Epitope Retrieval Solution	40	Dako Hercep Test	NEAT	Dako	20029667	30 Minutes	Visualization Solution	N	N	DAB

PR

Labs/Fields	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 minutes		16 1:100	Leica	6015355	32 minutes	OptiView	N	Y	DAB
102	DAKO PT - HIGH pH TRS	10/20/10		16 1:150	NOVOCAS TRA	6027462	30" RT	DAKO FLEX	NO	CUSO4	DAB+
106	CC1	64	PjR1294	1:150	Dako	10109383	32	Optiview	no	yes	DAB
109	HER HIGH pH (CC1)	36 MN	1294	1/50	DAKO	10092055	16 MN	ULTRAVIEW	N	Y	DAB
111	CC1	48 MN		16 1/80	LEICA	6027462	32 MN	OPTIVIEW	N	Y	COPPER
112	BOND ER2 pH9.0	12 minutes		16 RTU	Leica	43780	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
114	CC1	32		16 1/25	Novocastra	6027462	16	Optiview	N	Y	DAB
115	HER	30 mins	PjR 1294	1/50	Dako	10104594	20 mins	Envision Flex	N	N	DAB
116	CC1	64 MN	CLONE 16	1/100	LEICA	6027462	48 MN	ULTRAVIEW DAB	Y	.	DAB
119	HER/OC1	30 min	1E2.	pre-diluted	Ventana	FO9470	16 min	Ultraview	nil	nil	DAB
120	waterbath	20	PjR 636	Pre-dilute	Dako	10096659	20	Autostainer link48 - Envision Flex	Y	N	DAB
122	HER PH9	20		16 N/A	LEICA BIOSYSTEMS	29878	15 MN	POLYMER REFINE	N	N	DAB
123	CC1	64		16 1/25	LEICA	6027462	60 W	ULTRAVIEW	N	Y	DAB
124	CC1	30 min	1E2.	Predilute	Ventana	F07130	12	Ultra view	n	n	DAB
125	VENTANA CC1	36	1E2.	predilute	ROCHE	F07776	8	VENTANA ULTRAVIEW	N	Y	DAB
126	steam Citrate, ph 6.01	45 minutes	PjR 636	1:500	Dako	10098620	30 minutes	Envision + Mouse	n	n	DAB +
127	automated (CC1 on benchmark ultra)	36 minutes	1E2.	predilute	Ventana	F00392	8 minutes	ultraview detection kit	n	N	DAB
128	CC1	64 min	1E2.	Pre-dilute	Ventana	F05768	16 min	Ultraview	No	Yes	DAB
129	ER2 - high pH retrieval	20		16 1:400	Novocastra	Z050510	15	Bond Refine Detection Kit	N	N	DAB
132	FLEX HIGH PH	20	PR16	1:200	VECTOR	6024555	30	ENVISION FLEX	NONE	NONE	DAB
133	hier	64		16 1/25	Vector	6031757	60	Ultraview	n	n	DAB+
134	CC1	30	1E2.	RTU	ROCHE	F00392	8	ULTRAVIEW W	N	N	DAB
138	HER - EDTA hier	20	636 RTU		Dako	10106757	20	Polymer - Envision FLEX	N	N	DAB
139	automated	30 min.	1E2.	ready use	Ventana	F07130	32 min.	iview	no	no	dab
141	HER	30 min	1E2.	RTU	Ventana/Roche	F00392	12 min	ultraView	N	N	DAB
143	HER, automated online system	>30 but <45 min	1E2.	no	Confirm Ventana/Roche	F05768	24 min	Optiview detection kit	no	yes	DAB
145	CC1	32		16 1/100	NOVOCAS TRA	6027462	24	XT OPTIVIEW ihc v4	no	no	DAB
146	Flex TRS High	20	636 RTU		Dako	10101557	20	EnVision	N	N	DAB
147	HER, pH9 PT Link high	20		16 1:800	Novocastra	6027295	15	Polymer (Leica Refine)	N	N	DAB
149	pH	20	PjR636	RTU	Dako	10107676	20	Envision Flex	Yes	No	DAB
150	cc1	30min	1E2.	n/a	Ventana	F05768	20min	ultraview	n	y	DAB
151	Bond HER 1	20 min	1A6	1:200	NCL	6027295	15 min	Bond Refine Polymer	N	N	DAB
152	HER	60	1E2.	Pre-diluted	Roche Ventana	F05104	32	VIEW	N	Y	DAB

Labs/Fields	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	
153	HER CC1	32	1E2.		Predilute	VENTANA	F02791	24	OPTIVIEW	N	Y	DAB
155	CC1	30	1E2		Predilute	Ventana	F07130	32	Ultraview dab	n	y	dab
157	CC1	24 MIN.	1E2		FRE DILUTED	VENTANA	F05768	24 MIN.	OPTIVIEW/BENCHMARK XT	Y	Y	DAB
159	Flex TRS High	60 min total	PjR 636		none prediluted	Dako	10107676	30 min	Flex Ventana Ultraview	N	N	Flex Dab
160	EDTA pH8	36	1E2.		No	Ventana	?	8	Envision Flex	N	N	DAB
161	HER-High EDTA TRIS tampon	20 Minutes	PjR 636	RTU	DAKO	10106757	20 Minutes	Envision Flex	No	No	DAB	
162	CC1 Ventana	48 min		16 1:80	Leica	6028032	32 min	Optiview DAB, Ventana	N	N	Optiview DAB, Ventana	
164	ultraCC1	36	1E2.		predilute	Ventana	F07130	12	Ultraview	n	n	DAB
165	hier	30 min	1E2.		nil	ventana	105768	28min	ultraview	n	n	dab
167	CC1	30 min	1E2.		pre-diluted	F02791	Roche	8 min	Ultraview DAB kit	N	Y (copper)	DAB
168	HER	48	PjR636		RTU	Dako	10107676	20	Envision Flex +	N	Y	DAB
170	HER	20 min	PjR 636		ready to use	Dako	10106757	20 min	EnVison Flex	N	N	DAB
173	CC1 EDTA	30	1E2.		pre-diluted	Ventana	F00256	16	Ultraview polymer	N	Y	DAB
174												
175	HER	64	1E2.		pre-dilute	Roche	F09470	32	Ultraview	N	Y	DAB
177	CC1	32 min	PjR636		1:25	Dako	-	32 min	Ultraview	y	y	DAB
178	HER	32	1E2.		none	Ventana	n	16	Ultraview	n	n	DAB
183	Ultra CC1	36	1E2.		predilute	Roche	F02563	32	Ultraview	n	n	DAB
186	HER	20 MIN	FR88		1:100	BIOGENEX	MJ3280414	15 MIN	DAB BOND POLYMER	N	N	DAB
187	CC1	64	1E2		Predilute	E07088	F09470	12	Ultraview	None	None	Dab
188	HER ER2 BOND	20 MN		16	BOND RTU	LEICA	41887	15	BOND POLYMER REFINE KIT	N	N	DAB
189	CC1	64	1E2.		pre-dilute	Ventana	unknown	16	ultraView	N	N	ultraView DAB
190	CC1	32		16 1:50	NOVOCAS TRA	6027462	32	VIEW	N	N	DAB	
192	UltraCC1	36 minutes	1E2.		Ready to use	Ventana/Roche	F02563	16 minutes	VentanaUltraview DAB	N	Y (copper)	DAB
194	CC1	30		100	PREDILUTE	VENTANA	F05104	20 MINS.	VIEW KIT(AVIDIN BIOTIN)	N	Y	DAB
196												
198	Citrate pH 6.2 pressure cooker in microwave	5 min		16 1/600	Vector Laboratories	6024555	30 min	MACH 1 polymer	Y	Y	DAB	
199	HER	15		16 1:200	Leica	6027462	20	Refine	N	N	DAB	
202	citrate pH9.5 Leica	30 min	1.60E		RTU Leica	Leica	43163	15 min	Refine Detection kit Leica	no	no	dab
207	CC1-ON LINE	36 MIN	1E2.		PREDILUTE	VENTANA	F07776	16 MN	ULTRAVIEW W	N	N	DAB
209	HER	20mins at 97C and then 20mins cooling down to 85C	PjR 636		Pre dilute	Dako	10107676	20 mins	Envision plus	N	N	DAB
215	HER	64	1E2.		pre-dilute	ROCHE	F07130	16	Ultraview	N	N	DAB
216	HER	30	1E2.		PRE-DILUTE	VENTANA/ROCHE	F07130	24	AVIDIN/BIO TIN (VIEW DAB)	N	Y	DAB
217	CC1	64	1E2.		pre-dilute	Ventana	E09558	16	Optiview Mouse	No	No	DAB
221	pH6 Citrate Buffer	20 Minutes	1A6		1:200	Leica	6027462	30 Minutes	EnVision	N	N	DAB