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CIQC

Assessors' report for CIQC Run 55: Breast Module (ER, PR and HER2)

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Assessment performed: April 01, 2016, Saskatoon City Hospital

The expert assessment included evaluation of ER, PR, and HER2 immunoassays performed on the series of breast cancer cell lines with various levels of expression of ER, PR, and HER2. Cell lines offer a renewable resource for IHC external proficiency testing and, as such, CIQC has used them in a number of runs and anticipate doing so in the future. They provide a different “read-out” than the tissue micro-arrays we normally use, and we hope that the two types of runs will prove to be complementary.

The design of the proficiency testing sample, being composed of cell lines only, rather than the series of patients' tumours, was prepared with the purpose to analyse analytical sensitivity and specificity, rather than diagnostic sensitivity and specificity. In addition, the assessment also included evaluation of haematoxylin, which is essential when nuclear markers are performed as well as for the presence of cytoplasmic staining and/or floaters. These additional parameters are also very important because hematoxylin staining could completely obscure weak nuclear signals for ER and PR and the presence of “floaters” in the cell line-based proficiency testing suggests that the assay is using very harsh epitope retrieval protocols, which may result in tissue loss in small biopsy specimens or even larger tissue fragments if breast tissue is not optimally fixed.

It is expected that the laboratories that demonstrated unacceptable dark hematoxylin staining will change this as soon as possible. The results with hematoxylin were designated as “unacceptable dark” only when all expert assessors agreed that there is a serious problem with interpretation of results with ER and PR. The CIQC will continue to monitor the performance of the laboratories regarding the hematoxylin staining.

The presence of large number of floaters (tentatively agreed to be designated as such if more than 3 cell line cores were contaminated with cells from other cell lines) should initiate a review of IHC protocol and comparison with protocols from other laboratories that did not demonstrate any floaters. It is expected that in some cases excessive antigen retrieval will be identified as a cause, while it is entirely possible that in some cases no definite cause will be identified. The CIQC will continue to monitor for floaters in future runs that include cell line samples.

Currently there are no defined standards for analytical sensitivity and specificity of breast cancer markers. Proficiency testing runs that are using various cell lines have a potential in setting up the stage for development of a defined analytical standards. However, at this time, lacking firm defined values for cut off points, it is more useful and appropriate to show comparison of the achieved overall score of intensity of each laboratory with a reference to mean intensity score achieved by selected 6 major reference centers. The results for ER, PR, and HER2 intensity scores are shown in Figure 1, 2, and 3 respectively.

The intensity score was derived from expert assessment readout at which the following 4-tier scoring system was used: score 4 (>50% cells stain with $\geq 3+$ intensity), score 3 (>50% cells stain with $\geq 2+$ intensity), score 2 (>50% cells stain with $\geq 1+$ intensity), score 1 (there is <50% cells with $\geq 1+$ intensity), and score 0 (no positive cells present). During the evaluation, assessors were able to exclude staining (or lack of staining) of the floaters in order to achieve more objective readout of each cell line core. This is especially important for excluding false positive and false negative results secondary to contamination with the other cell line. All results of intensity scores are shown in Figure 1, 2, and 3. Each figure includes a horizontal line (so-called "reference line"), which represents a mean of the intensity scores achieved by the 6 major reference laboratories (national and international). Therefore, recommended intensity score should be close to the reference line.

CIQC recommends that the current results be interpreted with caution. The change of the protocol for Class 2 IHC, such as ER, PR, and HER2, should not be done without revalidation. If self-assessment revealed number of false-negative and/or false-positive results and the current analytical sensitivity and specificity appears very low or high compared to the mean of the six reference laboratories, the change of the protocol could be considered. In the first place, careful review of the laboratory protocol in parallel to the review of the protocols close to reference laboratory mean is suggested to evaluate if any major differences are noted. However, CIQC will also assess if the achieved results for analytical sensitivity and specificity impact diagnostic sensitivity and specificity of the laboratories by correlating this data set with the next run that will include patients' samples.

Q&A Run 55 Impact to Laboratory Quality

1. If hematoxylin seriously interferes with interpretation and the "intensities" are fine, what does that mean for the lab?

- If all assessors thought that hematoxylin seriously interferes with the readout, special attention was made to try to adjust for hematoxylin interference. However, it cannot be completely excluded that the intensity results may have been altered by the dark hematoxylin. Image analysis algorithm that is set up for the reference lab (with different hematoxylin) would likely cause larger error than the human eye that can adjust for interference.

2. What is the composition (and expected "expression levels") of the cell lines?

- As above, there are not set standards. Run 55 "standard" is the mean of the 6 reference laboratories.

3. Maybe the serious hematoxylin-offenders should be excluded from calculating intensity score?

- None of the reference laboratories that were used to calculate mean had a problem with hematoxylin. However, individual labs were not excluded. The assessors were careful to adjust their readout as much as possible.

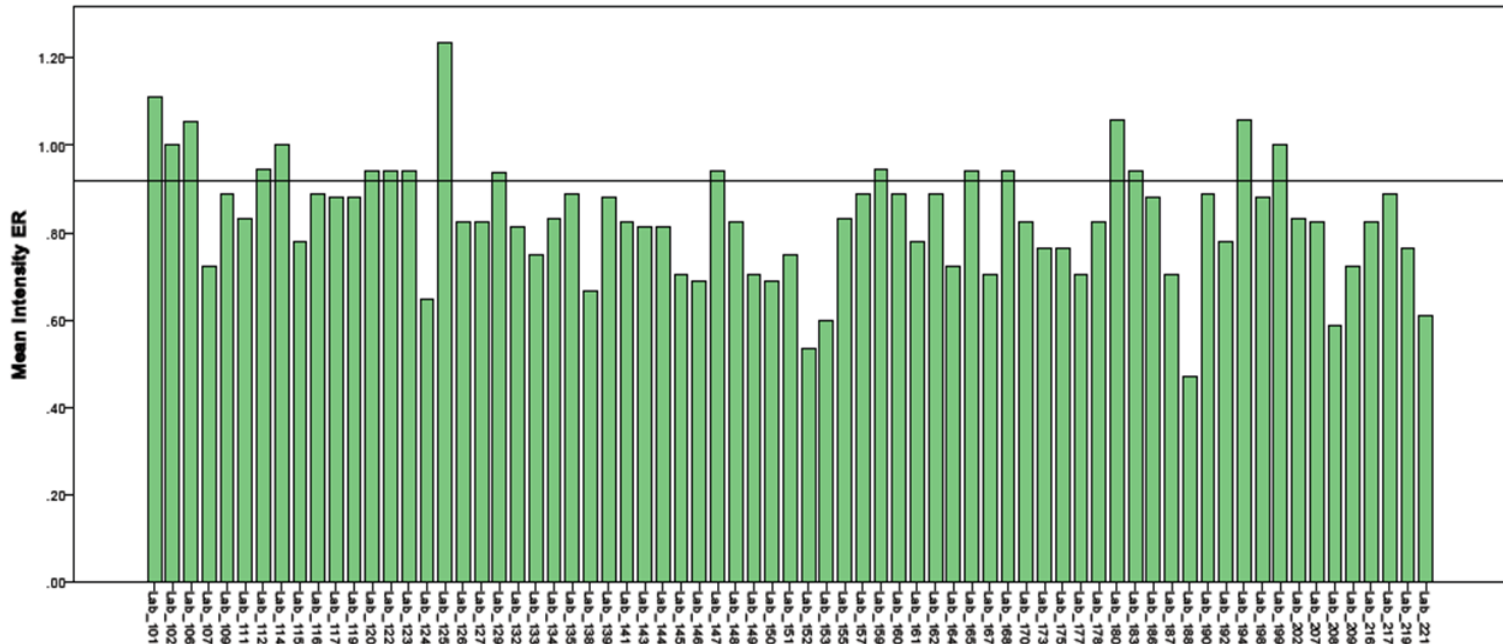
4. I do not know what constitutes a "pass". Did we pass?

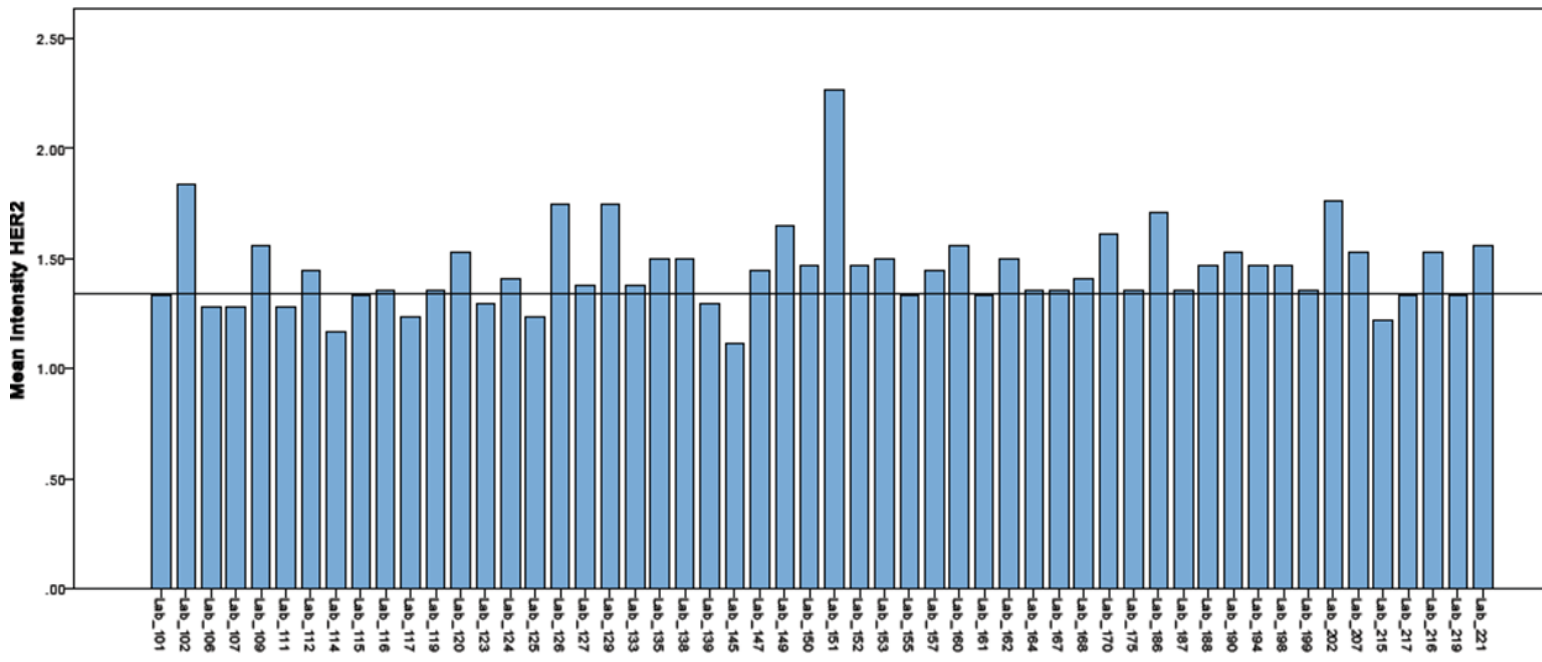
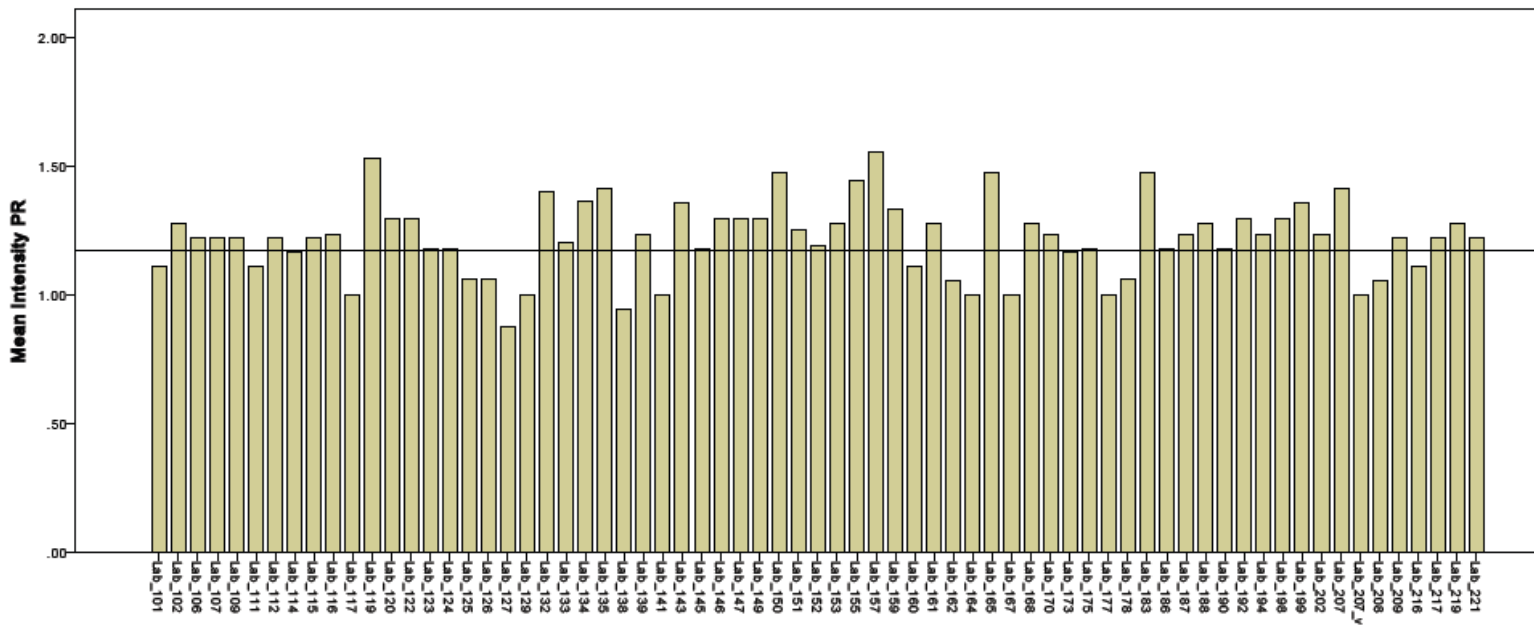
- Pass and fail cannot be determined with certainty with cell lines only. At the moment, only results with patients' samples can determine whether the lab will pass or fail. This run is used to assess differences in analytical sensitivity and specificity, not diagnostic sensitivity and specificity. Once we correlate how these fit with patients' samples results, we will be able to help laboratories finely tune their test and, hopefully, also finely monitor the trends. The laboratory should be concerned that the calibration is likely suboptimal if they stand out in bottom three or top three. After careful review of the bottom and top three laboratories, we can state that it is definitely possible to develop technically better protocols.

Detailed Data for Hematoxylin Intensity, Cytoplasmic signal, Floaters

Lab Number	Hematoxylin Intensity (ER slides)	Cytoplasmic signal (ER slides)	Number of Floaters (ER slides)
101	acceptable	yes	0
102	acceptable	yes	0
106	acceptable	no	1
107	acceptable	no	0
109	acceptable	no	0
111	acceptable	no	0
112	acceptable	no	0
114	acceptable	no	0
115	acceptable	yes	0
116	acceptable	yes	0
117	acceptable	no	0
119	acceptable	no	0
120	acceptable	yes	0
122	acceptable	yes	0
123	unacceptably dark	no	0
124	acceptable	yes	0
125	acceptable	no	0
126	acceptable	no	0
127	acceptable	no	3
129	acceptable	no	4
132	acceptable	yes	5
133	acceptable	no	2
134	acceptable	no	0
135	acceptable	yes	0
138	acceptable	yes	0
139	acceptable	no	0
141	acceptable	no	0
143	acceptable	no	1
144	acceptable	no	0
145	acceptable	no	0
146	unacceptably dark	yes	1
147	unacceptably dark	no	0
148	acceptable	no	0
149	acceptable	yes	0
150	acceptable	no	4
151	acceptable	yes	5
152	acceptable	no	0
153	acceptable	no	3
155	acceptable	no	1
157	acceptable	no	1
159	unacceptably dark	no	0
160	unacceptably dark	no	0
161	unacceptably	yes	0

	dark		
162	acceptable	no	0
164	acceptable	no	0
165	acceptable	no	0
167	acceptable	no	0
168	unacceptably dark	no	1
170	acceptable	yes	1
173	acceptable	no	0
175	acceptable	no	0
177	acceptable	yes	0
178	acceptable	no	0
180	acceptable	no	0
183	acceptable	no	0
186	acceptable	no	0
187	acceptable	no	0
188	acceptable	no	0
190	acceptable	yes	1
192	acceptable	yes	1
194	acceptable	no	0
198	unacceptably dark	no	0
199	acceptable	no	0
202	acceptable	no	0
207	acceptable	no	0
208	acceptable	no	0
209	acceptable	yes	0
216	acceptable	no	0
217	acceptable	no	0
219	acceptable	no	0
221	unacceptably dark	no	0





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 DAKO PT - 3in1 High pH TRS	10/20/10	SP1	1:50	Thermo	9101S1501E	32 minutes	OptiView	N	Y	DAB	
102	pH TRS performed onboard	64 min	SP1	predilute	DAKO Ventana Roche	10099710	30 MIN RT	DAKO FLEX	NO	CUS04	DAB+	
106	ultra cc1	36 min	SP1	pre-diluted	Ventana	F02226	32 minutes	Optiview ultraview DAB	no	no	DAB	
107	HIER high pH (CC1)	64 MIN	SP1	RTU	ROCHE/VENTANA	F04055	32 MIN	ULTRAVIEW	NO	YES	DAB	
111	CC1	36 MIN	SP1	PREDILUTE	VENTANA	F02583	32 MIN	ULTRAVIEW	N	COPPER	DAB	
112	Bond ER2 pH 9.0	20 minutes	SP1	1:150	Thermofisher	PH914231	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB	
114	CC1	32	SP1	1/50	Thermo Fisher	9101S1501E	16	Optiview	n	copper	DAB	
115	Envision Flex high pH	30min	EP1	Prediluted	Dako	10104563	30min	Envision Flex ultraView DAB	N	N	DAB	
116	CC1	64 MIN	SP1	RTU	VENTANA Roche/Ventana	F00389	64 MIN	DAKON	non	COPPER	DAB	
117	Cc1	32	SP1	aucune	Roche	F02226	38	Ultra View	N	CuSo4	Dab	
119	HIER	30 min	SP1	pre-dilute	Roche-Ventana	F04055	24 min	Ultraview	no	no	DAB	
120	waterbath	20	EP1	Ready to use	Dako	10100789	20	Flex polymer refine	N	N	DAB	
122	HIER ER2	20 min	SP1	N/A	Ventana	E08617	15min	view	n	n	DAB	
124	CC1 Omnis high pH	60 min	SP1	1/100	Cell Marque	1422401B	32	Dako EnV FLEX	n	n	DAB	
125	Steam Citrate, ph 6.01	30 min	SP1	predilute	Roche	F0283	30 min	Envision + Rabbit	no	no	DAB +	
126	AUTOMATED (CC1 ON BENCHMARK ULTRA)	64 MINUTES	SP1	PREDILUTE	NeoMarkers	9101S1501B	30 minutes	ULTRAVIEW DETECTION KIT	N	N	DAB	
127	CC1	64 min	100	Pre-dilute	Ventana	E07165	32 MINUTES	Ultraview	No	Yes	DAB	
128	ER2 - high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1501B	15	Bond Refine Detection Kit	N	N	DAB	
132	Envision Flex high pH	20 min	EP1	RTU	Dako	10098852	20	Envision Flex Ultraview polymer	N	N	DAB	
133	HIER	36 minutes	SP1	predilute	Roche	F00389	32 minutes	Ultraview Ventana	n	n	dab	
134	CC1	30 min	SP1	RTU	Roche	F00389	8 min	Ultraview	N	N	DAB	
135	buffer PH 9	20	SP1	1:100	Thermo Fisher	9101S1210L	15	Bond refined kit	N	N	DAB	
138	HIER	20	EP1	RTU	Dako	10104569	20	Polymer	N	N	DAB	
139	hier	30	SP1	ready use	Ventana	F04055	32	view	N	N	DAB	
141	HIER	30 min	SP1	Ready to use	Ventana/Roche	F00389	8 min	ultraView	N	N	DAB	
143	HIER, automated online system	>30 but <45 min	SP1	no	Confirm Ventana/Roche	E10753	16 min	Optiview detection kit	no	yes	DAB	
144	CC1	24 min.	SP1	1:50	ThermoScientific	910151406R	16 min.	Optiview	No	Yes-Copper	DAB	
145	CC1 Flex TRS high	32	SP1	1/100	CELL MARQUE	1316403E	28	OPTIVIEW ihc v4	NO	NO	DAB	
146	high	20	EP1	RTU	Dako	10095666	20	Envision Flex	n	n	DAB	
147	HIER, pH9 Ventana	20	SP1	1:150	ThermoScientific	9101S1501A	15	Bond Refine Polymer Ultraview	N	N	DAB	
148	CC1	36	SP1	RTU	Ventana	F04055	12	DAB	N	N	DAB	
149	PT Link high pH 97C	20	EP1	RTU	Dako	10102811	20	Envision Flex	No	No	DAB	
150	cc1	30min	SP1	n/a	Ventana	9101S1501A	16min	UltraView	n	y	DAB	
151	BUFFER pH9.0	20 MIN	SP1	1:100	THERMO FISHER	9101S1501A	15MIN	BOND REFINE	N	N	DAB	
152	CC1	60	SP1	Pre-diluted	Roche Ventana	E06575	16	VIEW	No	Yes	DAB	
153	HIER CC1	32	SP1	PRA% DILUA%	VENTANA	F00389	8	OPTIVIEW Ultraview	N	Y	DAB	
155	CC1	30	SP1	PRA% t A emploi	Ventana	F04055	40	Ultraview	n	n	dab	
157	CC1	24 MIN.	SP-1	PRE-DILUTED	VENTANA	E 02453	24 MIN.	OPTIVIEW, Benchmark XT	Y	Y	DAB	
159	high pH	40 min total	EP1	none, prediluted	DAKO	10102811	20 min.	FLEX	N	N	DAB	
160	EDTA pH8	36	SP1	No	Ventana	?	8	Ventana Ultraview	N	N	DAB	
161	HIER-High EDTA TRIS tampon	20 Minutes	EP1	RTU	DAKO	10100789	20 Minutes	Envision Flex	No	No	DAB	
162	Ventana	48 min	SP1	1:100	Thermo Scientific	9101-1501B	32 min	Optiview Ventana	-	-	DAB Ventana	
164	ultraCC1	36	SP1	predilute	VENTANA	F04055	8	OPTIVIEW	N	N	DAB	
165	hier	30 min	sp1	nil	ventana	f04055	28 min	ultraview	n	n	dab	
167	CC1	30 min	SP1	pre diluted	Roche	F00389	8 min	Ultraview DAB kit	N	Y (copper)	DAB	
168	HIER EDTA ph 9.0 HIER	48 min	SP1	1/100 ready to use	Cell Marque	1422401B	30	Envision Flex +	N	Y	DAB	
170	CC1	20	EP1	ready to use	Dako	10104569	20	Flex Dako Ultraview polymer	n	n	dab	
173	CC1-EDTA	30	SP1	prediluted	Ventana	E06774	16	Ultraview	N	Y	DAB	
174	HIER	36 mins	SP1	pre-dilute	Roche	F04055	32 mins	ultraview	N	Y-copper	DAB	
177	CC1	30	6F11	1:25	Novocastra	-	32	Ultraview	y	y	DAB	
178	HIER	32	SP1	none	Ventana	F02226	16	Ultraview	N	N	DAB	
180	CC1	32	SP1	RTU	VENTANA	E06774	16	OPTIVIEW	N	N	DAB	
183	ultra cc1	36 min	SP1	predilute	Roche	E10753	32 min	ultraview	n	n	DAB	
186	HIER	20 min	SP1	1:50	ThermoScientific	9101S1406H	15 min.	LEICA	N	N	DAB	
187	SP1	16	SP1	None	Roche	F02226	8	Optiview	N	N	DAB	
188	ER1 (on board BOND)	30	6F11	1:50	Leica Biosystems	6027092	30	Bond Polymer Refine Detection DS9800	N	N	DAB	
189	CC1	64	SP1	pre-dilute	Ventana	unknown	16	UltraView	N	N	DAB	
190	CC1	32	SP1	1:50	Ventana	F04055	32	Ventana Ultraview	N	N	DAB	
192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	F02226	16 minutes	Ultraview DAB	N	Copper	DAB	
194	CC1 Citrate pH 6.2 pressure cooker in microw ave	5 min	30	SP1	PREDILUTE	VENTANA	F02583	12 MINS.	VIEW AVIDIN/BIO TIN	Y	Y	DAB
198	HIER ER2 Bond solution	20	6F11	1/150	Leica	6027943	30 min	MACH 1 polymer	Y	Y	DAB	
199	ER2 Bond solution	30	6F11	RTU	Leica	40820	15	Refine	N	N	DAB	
202	ph9.5	20	6F11	1/300	Vector	6027092	15 min	Refine Detection kit	N	N	dab	
207	CC1	36 minutes	SP1	Prediluted	Ventana	F02583	16 minutes	Ultraview	N	Y	DAB	
208	HIER PH6	30	6F11	1/50	Leica	6027943	60	Envision Flex	N	N	Dab Flex	
209	HIER	20 mins at 97C and 20mins cooling to 85C	EP1	Pre dilute	Dako	10102811	20mins	Envision plus	N	N	DAB	
215	HIER	64	SP1	Predilute	Roche	E02453	32	Ultra	N	N	DAB	
216	HIER	30	SP1	PRE-DILUTE	VENTANA/ROCHE	F04055	24	AVIDIN/BIO TIN (VIEW DAB DETECTION KIT)	N	Y	DAB	
217	HIER (CC1)	64	SP1	Pre-dilute	Ventana	F04055	20	Optiview	N	Copper	DAB	
219	Heat induced epitope retrieval	36	SP1	Prediluted	Ventana	F04055	16	Ventana Ultraview Universal DAB Detection	N	N	DAB	

ER Protocols

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	16	1:200	Leica	6015355	32	OptiView	N	Y	DAB
102	DAKO PT 3in1 High pH TRS performed onboard	10/20/10	16	1:150	LEICA	6027462	30	DAKO FLEX	NO	CUSO4	DAB+
106	performed onboard	64 min	PgR 1294	1:250	Dako	10085593	32 minutes	Optiview	no	no	DAB
107	ultra cc1 HIER high pH	64 min	PgR1294	1:50	Dako	10104594	32 min	ultraview DAB	Y	Y	DAB
109	HIER high pH	36 MIN	1294	1/50	DAKO	10092055	16 MIN	ULTRAVIEW	NO	YES	DAB
111	CC1	48 MIN	16	1/80	LEICA	6027462	32 MIN	OPTIVIEW	N	COPPER	DAB
112	BOND ER2 pH 9.0	12 minutes	16	RTU	LEICA	25509	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
114	CC1	32	16	1/25	Novocastra	6027462	16	Optiview	N	Copper	DAB
115	Envision Flex high pH	30min	PgR1294	1/50	Dako	10092055	30	Envision Flex	N	N	DAB
116	CC1	64 MIN	CLONE 16	1/100	LEICA	6027462	48 MIN	ultraview DAB	YES	COPPER	DAB
117	CC1	12 min	NCL-L-PGR-312	1/200	Leica	6027462	38 min	Ultra View	N	CuSo4	Dab
119	HIER	30 min	100	pre-dilute	Roche-Ventana	F02563	16 min	UltraView	yes	yes	DAB
120	Waterbath	20	PgP 636	Ready to use	Dako Leica	10096659	20	Flex Polymer refine	Y	N	DAB
122	HIER ER2	20min	16	N/A	Leica Biosystems	29086	15min	iview	N	N	DAB
124	CC1	30 min	100	100	Ventana	F02563	20 min	ultraview universal DAB	n	n	Dab
125	Ultra CC1 STD Steam Citrate, ph 6.01	36 min	100	predilute	Roche	F05104	8 min	ultraview universal DAB	N	Y	DAB
126	AUTOMATED (CC1 ON BENCHMARK ULTRA)	45 minutes	636	1:500	DAKO	10098620	30 minutes	Envision + Mouse	no	no	DAB +
127	CC1	36 MINUTES	100	PREDILUTE	VENTANA	F00392	8 MINUTES	ULTRAVIEW DETECTION KIT	N	N	DAB
128	CC1	64 min	SP1	Pre-dilute	Ventana	F05768	16 min	ultraview	No	Yes	DAB
129	ER2 - High pH retrieval	20	16	1:400	Novocastra	Z050510	15	Leica Bond Refine Kit	N	N	DAB
132	Envision Flex High pH	20	Pr 16	1:200	Vector	6024555	30	Envision Flex	N	N	DAB
133	HIER	64 minutes	16	1/25	Vector	6031757	60 minutes	ultraview polymer	n	n	dab
134	CC1	320 min	100	RTU	Roche	F00392	8 min	Ventana Ultraview	N	N	DAB
135	buffer PH 9	20	16	1:400	Leica	1312100	15	Bond refined kit	N	N	DAB
138	HIER	20	PgR 636	RTU	Dako	10104336	20	polymer	N	N	DAB
139	Hier	30	100	ready use	Ventana	F05768	32	iview	N	N	DAB
141	HIER, automated online system	30 min	100	ready to use	Ventana/Roche	F00392	12 min	ultraView	N	N	DAB
143	HIER, automated online system	> 30 but <45 min	100	no	Confirm Ventana/Roche	E09558	24 min	Optiview detection kit XT	no	yes	DAB
145	CC1	32	16	1/100	NOVOCASTRA	6027462	28	OPTIVIEW the v4	NO	NO	DAB
146	Felix TRS High	20	636	RTU	Dako	10091119	20	Envision Flex	n	n	DAB
147	HIER, pH9 PT Link high pH 97 C	20	16	1:800	Novocastra	6027295	15	Bond Refine Polymer	N	N	DAB
149	HIER	20	PgR636	RTU	Dako	10105384	20	Envision Flex	Yes	No	DAB
150	cc1 BUFFER pH 6.0	30min	100	n/a	Ventana	0	20 min	UltraView	n	y	DAB
151	BUFFER pH 6.0	20 MIN	1A6	1:200	NCL Roche	6027295	15 MIN	BOND REFINE	N	N	DAB
152	CC1	60	100	Pre-diluted	Ventana	E06575	32	IVIEW	No	Yes	DAB
153	HIER CC1	32	F00392	PRA% DILUA% PRA% A l'emploi	VENTANA	100	24	OPTIVIEW	N	Y	DAB
155	CC1	30	100	100	Ventana	F02563	32	ultraview dab	n	n	dab
157	CC 1	24 MIN.	IE 2	PRE DILUTED	VENTANA	E 02707	24 MIN.	OPTIVIEW, Benchmark XT	Y	Y	DAB
159	high pH	40 min total	PgR636	prediluted	Dako	10105384	30 min	flex	N	N	DAB
160	EDTA pH8 HIER-Hier	36	100	No	Ventana	?	8	Ventana Ultraview	N	N	DAB
161	EDTA TRIS tampon	20 Minutes	PgR(636)	RTU	DAKO	10105384	20 Minutes	Envision Flex	No	No	DAB
162	CC1 Ventana	48 min	16	1:80	Leica	6020162	32 min	Optiview Ventana	-	-	DAB ventana
164	ultraCC1	36	100	predilute	VENTANA	F05768	36	OPTIVIEW	N	N	DAB
165	hier	30 min	100	n	ventana	f02563	24 min	ultraview Ultraview DAB kit	n	n	dab
167	CC1	30 min	100	pre diluted	Roche	F00392	8 min	Envision Flex +	N	Y (copper)	DAB
168	HIER	48	PgR636	RTU	Dako	10104336	20	Envision Flex +	N	Y	DAB
170	EDTA ph 9.0 HIER	20	PgR 636	ready to use	Dako	10104336	20	Envision Flex Dako	n	n	Dab
173	CC1-EDTA	30	100	prediluted	Ventana	F00256	16	ultraview polymer	N	Y	DAB
175	HIER	64 mins	100	Pre-dilute	Roche	F05768	32 mins	ultraview	N	Y-Copper	DAB
177	CC1	32	PgR636	1:25	Dako	-	32	ultraview y	y	y	DAB
178	HIER	32	100	none	Ventana	F00256	16	ultraview	N	N	DAB
183	Ultra CC1	36 min	100	predilute	Roche	F00256	32 min	ultraview	n	n	DAB
186	HIER	20 min.	PR88	1:100	Biogenex	MJ3290414	15 min.	LEICA	N	N	DAB
187	CC1	64.0	IE2	Pre-dilute	Roche	F02563	12.0	Optiview	N	N	DAB
188	ER2 (on board Bond)	20	16	Predilute	Leica Biosystems	41887	20	Bond Polymer Refine Detection DS9800	N	N	DAB Ultraview
189	CC1	64	100	pre-dilute	Ventana	unknown	16	UltraView	N	N	DAB
190	CC1	32	16	1:50	Novocastra	6027462	32	Ventana Ultraview	N	N	DAB
192	UltraCC1	36 minutes	100	Ready to use	Ventana/Roche	F00256	16 minutes	VentanaUltra view DAB	N	Copper	DAB
194	CC1 Citrate pH 6.2 pressure cooker in microwave	30	IE2	PREDILUTE	VENTANA	F00392	20 MINS.	VIEW(AVIDIN BIOTIN)	N	Y	DAB
198	HIER	5 min	16	1/600	Vector Laboratories	6024555	30 min	MACH 1 polymer	Y	Y	DAB
199	HIER ER2 Bond solution pH 9.5	15	16	1:200	Lecia	6027462	20	Refine Refine Detection kit Leica	N	N	DAB
202	CC1	36 minutes	100	Prediluted	ventana	F02791	16 minutes	ultraview	N	Y	DAB
208	HIER PH9	30	PGR636	Predilute	DAKO	1016757	60	Envision Flex	N	N	DAB Flex
209	HIER	20 mins at 97C and then 20mins cooling to 85C	PgR636	Pre dilute	Dako	10105384	20mins	Envision plus	Y	N	DAB
215	HIER	64	100	Predilute	Roche	E04944	16	ultraview	N	N	DAB
216	HIER	30	100	PRE-DILUTE	VENTANA/ ROCHE	F02563	24	VIEW DAB DETECTION KIT	N	Y	DAB
217	HIER (CC1)	64	IE2	Pre-dilute	Ventana	F02563	16	Optiview	N	Copper	DAB
219	Heat induced epitope retrieval pH6 Citrate Buffer	36	100	Ventana prediluted	Ventana	F02563	16	Ventana Universal DAB Detection Kit	N	N	DAB
221	Heat induced epitope retrieval pH6 Citrate Buffer	20	1A6	1:200	Leica	6027462	30	Mouse Envision	N	N	DAB

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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	SP3	1:200	Thermo	9103S1305I	32	OptiView	N	Y	DAB
102	DAKO PT 3in1 High pH TRIS performed onboard	10/20/10	SP3	1:750	THERMO	9103S1306 F	30 MIN RT	DAKO FLEX	NO	CUSO4	DAB+
106		36 min	4B5	predilute	Ventana Roche	F06373	16 min	ultraview	no	no	DAB
107	ultra cc1	36 min	4B5	pre-diluted	Ventana	F04797	8 min	ultraview DAB	N	Y	DAB
109	HIER high pH	36 MIN	4B5	RTU	ROCHE/VENTANA	F03787	16 MIN	ULTRAVIEW W	NO	YES	DAB
111	CC1	36 MIN/RT	4B5	PREDILUTE	VENTANA	F04797	32 MIN	ULTRAVIEW W	N	COPPER	DAB
112	BOND ER2 pH9.0	20 minutes	4B5	1:3 ratio of the RTU	Ventana	E00842	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
114	CC1	16	SP3	1/200	Thermo Fisher	9103S1202 E	16	Optiview	N	Copper	DAB
115	Herceptest Epitope Retrieval Solution	40	Herceptest Her2 protein	Prediluted	Dako	20028925	30	Herceptest kit	N	N	DAB
116	CC1	40 MIN	SP3	1/100	THERMOSCIENIFIC	1306 F	36 MIN	Optiview DAB	NON	YES	DAB
117	Cc1	16	(4B5)	aucune	Roche /Ventana	F04797	38	ultra View	N	CuSo4	Dab
119	HIER	30 min	4B5	pre-dilute	Roche-Ventana	F04797	16 min	UltraView	no	no	DAB
120	Waterbath	40	Herceptest	Ready to use	Dako	20022527	30	Hercep test	N	N	DAB
124	CC1	30 min	4B5	PrÃ©-dilua	Ventana	F04797	12	Ultra view ultraview Universal DAB	n	n	Dab
125	Ultra CC1 STD	36 min	4B5	predilute	Roche	F05081	16 min	Envision + Rabbit	no	no	DAB +
126	Steam TRIS, ph 10.01	55 minutes	SP3	1:100	Thermo Scientific (NeoMarkers)	9103S1509 8B	30 minutes	Envision + Rabbit	no	no	DAB +
127	AUTOMATED (CC1 ON BENCHMARK ULTRA)	36 MINUTES	4B5	PREDILUTE	VENTANA	F05081	24 MINUTES	ULTRAVIEW DETECTION KIT	N	N	DAB
129	ER1 - low pH retrieval	20	Cerb b2	1:600	Dako	86782	15	Leica Bond Refine Kit Ultraview polymer	N	N	DAB
133	HIER	36 minutes	4B5	predilute	Roche	F04797	24 minutes	Bond refined kit	N	N	DAB
135	buffer PH 6	20	polyclonal	1:700	Dako	81393	15	Bond refined kit	N	N	DAB
138	HIER	40	HercepTest	RTU	Dako	20023024	30	Polymer	N	N	DAB
139	Hier	30	4B5	ready use	Ventana	F04797	48	view XT OPTVIEW IHC v4	N	N	DAB
145	CC1	32	SP3	1/300	CELL MARQUE	1332302A	16	Bond Refine Polymer	N	N	DAB
147	HIER, pH9	20	SP3	1:50	ThermoScientific	QC1993201	15	EnVision Flex	Yes	No	DAB
149	PT Link low ph 97 C	20	Poly R	RTU	Dako	2022527	20	UltraView BOND REFINE	N	N	DAB
150	cc1 BUFFER pH 6.0	30min	SP3 POLYCLONAL	1/100	NeoMarkers	0	20 min	UltraView BOND REFINE	n	y	DAB
151		20 MIN		1:500	DAKO	20002512A	15 MIN		N	N	DAB
152	CC1	60	4B5	Pre-diluted	Roche Ventana LAB VISION	F05081	32	VIEW	No	Yes	DAB
153	HIER CC1	32	SP3	1/100	THERMO FISHER	9103S 1407D	32	OPTVIEW DAB	N	Y	DAB
155	CC1	30	4B5	PrÃ©-Ã l'emploi	Ventana	F03787	32	Ultraview dab OPTVIEW, Benchmark XT	n	n	dab
157	CC 1	24 MIN.	4B 5	PRE DILUTED	VENTANA	E 04951	24 MIN.		Y	Y	DAB
160	EDTA pH8	36	4B5	No	Ventana	?	12	Ventana Ultraview	N	N	DAB
161	Herceptest epitope	40 Minutes	Rabbit anti-human HER2 protein	RTU	DAKO	20026169	30 Minutes	Herceptest visualization	No	No	Herceptest DAB chromogen
162	CC1 Ventana	32 min	4B5	RTU	Ventana	F05081	20 min	UltraView - Ventana	-	-	DAB Ventana
164	ultraCC1	36	4B5	predilute	VENTANA	F04797	12	OPTVIEW	N	N	DAB
167	CC1	30 min	4B5	pre diluted	Roche	F04797	8 min	Ultraview DAB kit	N	Y (copper)	DAB
168	Cell Conditioning	30	4B5	RTU	Roche	F03787	16	Ultraview DAB	N	Y	DAB
170	citrate ph 6.0 HIER	40	Her 2	ready to use	Dako	20022527	30	Envision Flex dako	n	n	DAB
175	HIER	36 mins	4B5	Pre-dilute	Roche	F05081	16 min	ultraview	N	Y-copper	DAB
181	CC1 on board	30 minutes	4B5	pre-diluted	Ventana	F02223	16 minutes	Ventana Ultraview DAB	N	Y	DAB
186	HIER	20 min.	polyclonal	1:400	DAKO	20002512	15 min.	LEICA	N	N	DAB
187	CC1	16.0	4B5	Ready to use	Roche	F04797	24	Optiview BOND Oracle IHC System for HER2	N	N	DAB
188	ER1 (on board BOND) 97 Deg C	25	CB11	Predilute	Leica Biosystems	40801	30		N	N	DAB
189	CC1	32	4B5	Pre-dilute	Ventana	unknown	16	UltraView Ventana	N	N	UltraView DAB
190	CC1	32	SP3	1:50	Thermo	9103S1306 F	40	VIEW(AVIDIN BIOTIN)	N	Y	DAB
194	CC1	30	4B5	PREDILUTE	VENTANA	F05081	12 MINS		N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Ventana/Roche	F04797	20 min	Ultraview	N	Y	DAB
199	HIER	30	CB11	RTU	Lecia	41313	30	Oracle	N	N	DAB
202	DAKO Citrate Buffer	40 min	Her2	RTU from Herceptest kit	DAKO	20026169	30 min	Herceptest kit	N	N	DAB
207	CCI	36 min	4B5	prediluted	Ventana	F06373	16 minutes	Ultraview	N	Y	DAB
215	HIER	36	4B5	Predilute	Roche	E04951	12	Ultra	N	N	DAB
216	HIER	30	4B5	PRE-DILUTE	VENTANA/ROCHE	F05081	32	AVIDIN/BIO TIN (VIEW DAB DETECTION KIT)	N	Y	DAB
217	HIER (CC1)	32	4B5	Pre-dilute	Ventana	F03192	20	Optiview Ventana Ultraview Universal DAB Detection Kit	N	Copper	DAB
219	Heat induced epitope retrieval	36	4B5	Ventana prediluted	Ventana	F04797	16		N	N	DAB
221	Dako Visualization Solution	40	Dako Hercep Test	NEAT	Dako	20027207	30	Dako Kit	N	N	DAB

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