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Assessors' report for cIQc Run 39: Breast Module (ER, PR and HER2) (July 2014)

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Assessment performed on Wednesday, November 19, 2014 at Abbotsford Regional Hospital, British Columbia

Overview

Participating laboratories were asked to stain a tissue microarray consisting of formalin-fixed, paraffin-embedded cell blocks from nine breast cancer lines. Two cores from each cell line were included on the tissue microarray. ER, PR and HER2 mRNA expression levels were previously quantified by RT-PCR, providing an independent method of assessment to be used as a reference (R1) in the study.

Slides from Labs 141, 167 and 207 were not returned to cIQc in time for the assessment meetings. Available slides from all other participating labs were blindly reviewed by cIQc assessors. Independent review led to occasional alteration of original self-reported results for discordant cores due to 1) an obvious data entry error, 2) contamination from potential transfer of cells during TMA construction and sectioning, or 3) a core score was deemed to be discordant between self-assessment and final cIQc review and re-classified as either positive or negative.

ER: Core 4 showed weak positive staining, making it the best test of staining sensitivity on the array. Most labs had optimal or adequate staining. Assessors noted that labs observing false negative staining in Cores 4 and 13 reported use of the Ventana iView detection system. Similar to Run 36, false positive results were observed when using the Leica Bond-Max system with 6F11 antibody. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Optimal	
102	Optimal	
103	Optimal	
105	Optimal	
106	Optimal	More intense staining compared other labs, reviewers considered it exceptionally nice
107	Optimal	
109	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
115	Optimal	



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116	Optimal	
117	Optimal	Results not submitted online, but slide was available for cIQc assessment.
119	Adequate	Overall weak staining
120	Optimal	
122	Optimal	
123	Adequate	Dark counterstain, weak staining overall
124	Adequate	Core 4 and 13 were false negatives
125	Optimal	
126	Optimal	
127	Optimal	
128	Optimal	
129	Adequate	Core 4 and 13 were false negatives
132	Optimal	
133	Optimal	Slightly weak staining
134	Optimal	
135	Optimal	
138	Optimal	
139	Adequate	Weak staining, false negative in Core 4
141	--	Slide not submitted for review.
143	Adequate	Cytoplasmic staining masking weak nuclear staining in Core 4
144	Optimal	
145	Optimal	
146	Optimal	
147	Optimal	
148	Optimal	High number of uninterpretable cores due to too few cells
149	Optimal	



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150	--	Unlabeled slide submitted for review.
151	Optimal	
153	Optimal	
155	Optimal	
157	Optimal	
159	Optimal	
160	Optimal	Dark counterstain that can interfere with interpretation of nuclear staining
161	Optimal	
162	Optimal	
163	Optimal	
164	Optimal	
165	Optimal	
167	--	Slide not submitted for review.
168	Suboptimal	Very weak staining in Cores 7 and 18; Cores 4 and 13 were false negatives
170	Optimal	
173	Optimal	
175	Optimal	
177	Adequate	Weak staining, especially in Cores 4 and 13
178	Adequate	Cores 4 and 13 were false negatives
180	Optimal	
181	Optimal	Results not submitted online, but slide was available for cIQc assessment.
183	Optimal	Very dark counterstain, but still able to interpret staining
184	Optimal	
186	Optimal	
187	Optimal	
188	Adequate	Overall increased cytoplasmic background staining, Core 13 was a false negative
189	Optimal	



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190	Optimal	
191	Optimal	
192	Optimal	
194	Optimal	
196	Optimal	
198	Optimal	
199	Adequate	Core 5 and 10 were false positives
202	Optimal	Slightly weak staining
207	--	Slide not submitted for review.
208	Optimal	
209	Optimal	

*Based on cIQc review of staining

PR: Unlike previous challenges, PR staining showed little to no variability in this challenge. Core 7 showed weak positive staining, making it the best test of staining sensitivity on the array. All labs had either optimal or adequate staining. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Optimal	
102	Optimal	
103	Optimal	
105	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
111	Adequate	Overall more background staining compared to other labs
112	Optimal	
114	Optimal	
115	Optimal	
116	Optimal	



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117	Optimal	Results not submitted online, but slide was available for cIQc assessment.
119	Optimal	
120	Optimal	
122	Optimal	
123	Optimal	
124	Optimal	Large number of uninterpretable results that were interpreted as negative on review
125	Optimal	
126	Optimal	
127	Optimal	
128	Optimal	
129	Optimal	
132	Optimal	
133	Adequate	Slightly weak staining
134	Optimal	
135	Optimal	
138	Optimal	
139	Optimal	
141	--	Slide not submitted for review.
143	Optimal	
145	Optimal	
146	Optimal	
147	Optimal	
149	Optimal	
150	--	Unlabeled slide submitted for review.
151	Optimal	
153	Optimal	



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155	Optimal	
157	Optimal	
159	Optimal	
160	Optimal	
161	Optimal	
162	Optimal	
163	Optimal	
164	Optimal	
165	Optimal	
167	--	Slide not submitted for review.
168	Optimal	
170	Optimal	
173	Optimal	
175	Optimal	
177	Adequate	Background staining observed in negative cores and overall weaker staining in positive cores
178	Optimal	
181	Optimal	Results not submitted online, but slide was available for cIQc assessment.
183	Optimal	
184	Optimal	
186	Optimal	
187	Optimal	
188	Optimal	
189	Optimal	
190	Optimal	
191	Optimal	
192	Optimal	
194	Optimal	



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196	Optimal	
198	Optimal	
199	Optimal	
202	Optimal	
207	--	Slide not submitted for review.
208	Optimal	
209	Optimal	

*Based on cIQc review of staining

HER2: Overall, the quality of HER2 staining was excellent. In general, a qualitative assessment of staining by participating labs was performed and cIQc assessors primarily focused review on HER2 3+ cores (Cores 3,4,8 12,13 and 17).

Lab	IHC Status*	cIQc Comments
101	Optimal	
102	Optimal	
103	Optimal	
105	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
115	Optimal	
116	Optimal	
117	Optimal	Results not submitted online, but slide was available for cIQc assessment.
119	Adequate	Overall weak staining; Core 3 was a false negative but assessors noted a potential artifact (possibly a bubble during primary Ab incubation) encompassing both Cores 3 and 4.
120	Optimal	



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123	Optimal	Technically optimal but interpretive differences were noted by assessors
124	Optimal	
125	Optimal	
126	Optimal	
127	Optimal	Results not submitted online, but slide was available for cIQc assessment.
129	Optimal	
133	Optimal	
135	Optimal	
138	Optimal	
139	Optimal	
145	Optimal	
147	Optimal	Slight background staining
149	Optimal	
150	Optimal	
151	Optimal	
152	Optimal	
153	Optimal	
155	Optimal	
157	Optimal	
160	Optimal	
161	Optimal	
162	Optimal	
164	Optimal	
167	--	Slide not submitted for review.
170	Optimal	
175	Optimal	
181	Optimal	



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186	Optimal	
187	Optimal	
188	Optimal	
189	Optimal	
190	Optimal	
191	Optimal	
194	Optimal	
198	Optimal	
199	Optimal	Slightly weak/pale staining compared to other labs
202	Optimal	
207	--	Slide not submitted for review.

*Based on cIQc review of staining

Revised Garrattograms for ER, PR and HER2 are provided in Supplementary Figures 1 to 3. Supplementary Tables 1 to 3 summarizing staining protocols can also be found at the end of this document. Your regular participation in cIQc is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Figure S3. Revised Garrattogram after cIQc HER2 assessment.

Lab/ Core	101	102	103	105	106	107	109	111	112	114	115	116	119	120	123	124	125	126	129	133	135	138	139	145	147	149	150	151	152	153	155	157	160	161	162	164	167*	170	175	181	186	187	188	189	190	191	194	198	199	202	207*	R1														
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	1	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	N										
2	N	N	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	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	1	N	N	N									
3	3	3	3	3	3	3	U	3	3	3	3	3	N	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	2	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3									
4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3									
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	1	N	N	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	1	N	N	N						
6	N	N	N	N	N	N	U	U	N	N	U	N	N	N	N	N	N	N	N	N	N	U	U	U	U	U	U	N	N	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	1	N	N	N								
7	N	N	1	N	N	N	N	N	N	N	N	1	1	1	N	N	N	N	N	1	N	1	1	N	N	1	1	N	2	N	N	N	N	1	N	N	1	1	1	1	1	N	2	N	N	1	N	N	N	N	N	N	N	N	1	N	N	N								
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3								
9	N	1	2	1	N	1	1	1	1	N	1	1	2	1	1	N	2	1	2	1	2	2	2	1	1	2	2	1	2	1	1	1	N	2	1	1	1	2	2	2	N	2	N	N	1	N	2	N	N	2	2	1	N	N	2	2	1	N								
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	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	N							
11	N	N	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	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N						
12	3	3	3	3	3	3	3	3	3	3	3	3	2	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3							
13	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3						
14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	U	N	N	N	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	N	N	N	N	N			
15	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	1	N	N	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	N	N	N	N	N	N		
16	N	N	1	1	N	N	N	U	N	N	U	N	U	N	1	N	1	1	1	1	1	1	1	N	N	2	1	N	2	N	N	N	N	N	N	N	1	N	1	1	1	1	N	N	2	N	1	1	N	2	1	1	1	1	N	2	1	1	1	N	N	N				
17	3	3	3	3	3	2	3	3	3	3	3	3	2	3	2	3	3	3	3	3	3	3	U	U	U	U	3	3	3	3	2	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3			
18	N	2	2	1	1	1	1	1	1	N	2	1	1	1	1	1	2	2	2	2	2	2	2	1	1	2	2	1	2	1	N	1	2	2	1	1	1	2	2	2	2	1	2	1	2	1	2	1	2	1	2	2	2	2	2	2	2	2	2	N	N	N	N	N	N	N

Table S1. Reported ER staining protocols.

Lad ID	Clone	Dilution	Supplier	Ab Lot #	Ab Incubation Time	Ag Retrieval	Detection	Enhancement	Chromogen
101	SP1	1:50	Lab Vision	9101S1210I	32 minutes	CC1 32 minutes	Optiview	Copper	DAB
102	SP1	1/35	THERMO	9101S1308F	30* RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CUS04	DAB+
103	SP1	Prediluted	Ventana	D08604	16 minutes	CC1 64	Ultra View	Copper	DAB
105	SP1	1:50	Thermoscientific	9101S1210M	1 hour no heat	CC1 standard	Ultraramp	no	DAB
106	6F11	1:100	Leica	6022580	60 minutes	3 min, 115C Tris	Elite	none	DAB
107	SP1	Pre-diluted	Ventana	D11267	16 min @ 37C	Ultra CC1 - 36 min	UltraView DAB	NA	DAB
109	6F11	1/20	VECTOR	6027092	44 MINUTES	ULTRA CC1 64MIN@95	ULTRAVIEW DAB KIT	COPPER	DAB
111	SP1	Predilute	Ventana	D08925	32 min. RT	CC1 - 36 min	2-step multimer	Copper	DAB
112	SP1	RTU	VENTANA	D06729	20 MINUTES	CC1 MILD	ULTRAVIEW	COPPER	DAB
114	SP1	1/50	Thermo	9101S1308F	16mins	CC1 32 mins	OptiView	Non	DAB
115	SP1	Prediluted	Ventana	E00035	32 min	CC1	IVIEW DAB	COOPER	DAB
116	SP1	RTU	Ventana	D08925	32 min	CC1 64 min	UltraView DAB	no	DAB
119	SP1	Prediluted	Ventana	--	--	HIER/CC1	utraview	-	DAB
120	EP1	pre diluted	Dako	10084297	20 min	yes- 20 min	Dako Autostainer Link 48	Flex 20	Dab
122	SP1	NA	Ventana	E00035	15min	pH9 (ER2)	Polymer refine	N/A	DAB
123	SP1	predilute	Ventana/Roche	D11267	32	CC1 for 30 min	ultraview	copper	DAB
124	SP1	1/100	Cell Marque	1316403B	32 min	CC1 60 min	IView	none	DAB
125	SP1	predilute	Roche	D11267	15 min	Leica Bond ER2-20	Leica Bond Polymer Refine	na	DAB
126	SP1	1:200	Thermo Scientific	9101S12090	30min	Pressure Cooker	Rabbit EnVision	N/A	DAB
127	SP1	predilute	Ventana		32 mins.	36 mins.	ultraView DAB	none	DAB
128	SP1	Prediluted	Ventana	serial #121402	16min	standard 1	ultra-view	copper	DAB
129	SP1	1:50	Thermo Fisher	9010S1305F	15 min	20 min	Bond Refine	no	DAB
132	6F11	1:80	Vector	6029951	30 minutes	Flex High pH	Flex Plus	None	DAB
133	SP1	Predilute	Ventana	D08925	24 minutes	36 minutes CC1	UltraView Ventana	none	Dab
134	SP1	Ready to Use	Ventana/Roche	D06729	8 min	HIER 30 min	ultraView	None	DAB
135	SP1	1:50	Thermol Fisher	9101S1210L	15 min	Bond ER2 20 min	Bond polymer refined kit	none	DAB
138	EP1	RTU	Dako	10086340	20 minutes	High pH HIER	Dako Envision FLEX	none	DAB
139	SP1	ready use	Ventana	E00035	32 min	CC1	Iview	no	DAB
141	SP1	Ready to Use	Ventana/Roche	D06729	8 minutes	HIER 30 minutes	ultraView	None	DAB
143	sp1	no	confirm ventana	D07686	32 min	CC1 60 min	I view DAB detection kit	no	no
144	AP1	1:50	ThermoScientific	9101S1308F	16 minutes	Ultra CC1	Optiview	Copper	DAB
145	SP1	1/100	CELL MARQUE	1316403F	36 mins	CC1 32mins	XT OPTIVIEW DAB IHC v4	no	DAB
146	EP1	RTU	Dako	10081265	20 min	Flex TRS High	EnVision Flex Peroxydase	non	DAB
147	SP1	1:50	FISHER	9101S1308A	15 mins	Bond ER2 (pH9)	Bond Refine	N/A	DAB
148	SP1	RTU	VENTANA	E00035	8 min	CC1 36min	UltraView	none	DAB
149	EP1 IR084	RTU	Dako	10087383	20 min	PT Link ph 9 98 C 20 min	Envision Flex	No	DAB
150	sp1	n/a	ventana	0	32min	cc1	ultraview	copper	dab
151	SP1	1:100	THERMO FISHER	9101S1305F	15MIN	pH9.0/20 MIN	POLYMER	NO	DAB
153	SP1	S/O	Ventana	E0035	12 minutes	CC1 30 min	UltraView DAB	Ultra View Copper	DAB
155	SP1	predilute	Ventana	D09103	40 min	CC1	UltraView dab	UV copper	Dab
157	SP-1	PRE DILUTED	VENTANA	--	--	CC1 30 MIN	OPTIVIEW BENCHMARK XT	YES	YES
159	EP1	prA@diluted	DAKO	10088554	20'	High	FLEX	none	DAB
160	SP1	No	Ventana	D08925	8 min	EDTA pH8	Ventana UltraView	No	DAB
161	EP1	RTU	DAKO	10088554	20 minutes	High EDTA TRIS buffer	Envision Flex	no	DAB
162	SP1	1:100	Thermo Scientific	9101s1308A	32 min	CC1 48 min	OptiView DAB	-	OptiView DAB
163	SP1	Prediluted	Benchmark Ventana	E02453	32 min	Hier	Peroxydase	Copper	DAB
164	SP1	predilute	Ventana	E00035	8 minutes	CC1	UltraView	no	DAB
165	sp1	nil	ventana	E0035	28 min	cc1	ultraview dab	ultraview	dab
167	SP1	predilute	Ventana (Roche)	D04922	8 mn	CC1 (Roche)	DAB	Copper	DAB
168	SP1	1/200	CellMarque	1231906B	20 min.	High pH	Envision Flex	Rabbit Linker	DAB
170	Ep1	ready to use	Dako	10088554	20 min	HIER ph high	Envision Flex Dako	no	DAB
173	SP1	Pre-diluted	Ventana	d09103	16 minutes	CC1	UltraView	Polymer	DAB
175	SP1	predilute	Ventana	E00035	32	CC1	UltraView	Copper	DAP
177	6F11	1 : 25	Novocastra	.	32	yes	UltraView	yes	DAB
178	SP1	none	Ventana	na	16 min.	32 min.	UltraView	none	DAB
180	SP1	RTU	Ventana	D07686	16	CC1 Mild	iView DAB	none	DAB
183	SP1	PREDILUTE	VENTANA	DO4210	32 MIN	ULTRA CC1	ULTRAVIEW	NONE	DAB
184	EP1	RTU	Dako	10085567	20 min	HIER	Envision Flex	NA	DAB+
186	SP1	1:50	Thermoscientific	9101S1308A	15'	HIER EDTA PH9	Bond refine detection kit	None	DAB
187	SP1	Predilute	Roche	E00035	8.0 Mins	CC1	Optiview	None	DAB
188	6F11	1/50	Leica Biosystems	Current	20 mins	ER1 20 mins	Refine Kit	None	DAB
189	SP1	pre-dilute	Ventana	??	16 min./37A°	CC1 Standard	UltraView	None	UltraView DAB
190	no change	no change	no change	no change	no change	no change	no change	no change	no change
191	SP1	RTU	Roche	D06729	16'	CC1	ultraview	none	DAB
192	SP1	READY TO USE	VENTANA	D09103	16 MIN	ULTRA CC1 (36 MIN)	Ventana UltraViewDAB	Copper	DAB
194	SP1	PREDILUTE	VENTANA	D08925	12 MINS.	CC1	AVIDIN BIOTIN(IVIEW)	COPPER	DAB
196	SP1		VENTANA	D07686	8 MINUTES				DAB
198	6F11	1/100	Novocastra/Leica	6022580	30min RT	Citrate pH 6.2	Polymer- MACH 1	Copper Sulphate	DAB
199	6F11	RTU	Leica Microsystems	25434	15 mins	hier (Bond ER-2)	Polymer-Bond Refine	None	DAB
202	6F11	1/300	vector	6018294	15 min	er2 for 20 min	Leica refine detection kit	none	dab
207	SP1	PREDILUTED	VENTAN	D112367	16 MINUTES	CC1-36	ULTRAVIEW	NONE	DAB
208	6F11	1:50	Leica	6018835	60 MIN	PH 6.0	Envision Flex	-	DAB flex
209	EP1	NA	Dako	10088554	30mins	20 mins in Tris/ EDTA at 97C and cool down for 20mins to 85C	Polymer	Non	DAB

Table S2. Reported PR staining protocols.

Lab ID	Clone	Dilution	Supplier	Ab Lot #	Ab Incubation Time	Ag Retrieval	Detection	Enhancement	Chromogen
101	Clone 16	1:100	Novocastra	6015355	32 minutes	CC1 32 minutes	Optiview	Copper	DAB
102	16	1/150	NOV/CASTR	6011269	30' RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CU504	DAB+
103	100	Prediluted	Ventana	D07381	16'	CC1 64'	Ultra View	Copper	DAB
105	PgR636	1:200	Dako	10085019	1 hour no heat	CC1 standard	DABMAP	Signalstain enhancer	DAB
106	PgR 1294	1:1500	Dako	10076605	60 minutes	3 min, 115C, Tris	Elite	none	DAB
107	PgR 1294	1:50	Dako	10085593	32 min @ 37C	Ultra CC1 - 64 min	UltraView DAB	Ventana Amplification	DAB
109	16	1/100	VECTOR	6020167	32 MINUTES	ULTRACC1 -64MINUTES@95	ULTRAVIEW DAB KIT	COPPER	DAB
111	16	Predilute	Leica	6027464	32 min , RT	CC1-36 min	2-step Multimer	Copper	DAB
112	100	RTU	VENTANA	D03286	20 MINUTES	CC1 MILD	ULTRAVIEW	COPPER	DAB
114	16	1/25	Leica	RM-9103-S	16mins	CC1 32 mins	OptiView	Non	DAB
115	IE2	Prediluted	Ventana	D09634	32 min	CC1	IVIEW DAB	COOPER	DAB
116	clone 16	1/100	Leica	lot 6015355	48 min	CC1 64 min	UltraView DAB	yes	DAB
117									
119	16	Prediluted	Ventana	--	--	HIER/CC1	uutraview	-	DAB
120	PgR 636	pre diluted	Dako	10083456	20 min	yes - 20 min	Dako Autostainer Link 48	Flex+20	Dab
122	16	NA	NovaCastra	19169	15min	pH9 (ER2)	Polymer refine	N/A	DAB
123	16	1/50	Vector	6024555	32	CC1 for 60 min	ultraview	copper	DAB
124	IE2	none	Ventana	D09110	20 min	CC1 30 min	IView	none	DAB
125	100	1/4 of predilute	Roche	D09110	15 min	Leica Bond ER2-20	Leica Bond Polymer Refine	na	DAB
126	PgR 636	1:500	DAKO	10070290	30min	Pressure Cooker	Mouse EnVision	N/A	DAB
127	100	predilute	Ventana		8 mins.	36 mins.	ultraView DAB	none	DAB
128	IE2	Prediluted	Ventana	serial #131752	16min	Standard 1	ultra-view	copper	DAB
129	16	1:400	Novacastra	Z05010	15 min	20 min	Bond Refine	no	DAB
132	16	1:200	Vector	6020167	30 Minutes	Flex High pH	Flex Plus	None	DAB
133	16	1/50	Vector	6020167	32 minutes	64 minutes.CC1	UltraView Ventana	none	Dab
134	100	Ready to Use	Ventana/Roche	D05144	12 min	HIER 30 min	ultraView	None	DAB
135	16	1:400	Leica	1312100	15 min	Bond ER2 20 min	Bond Polymer refined kit	none	DAB
138	636	RTU	Dako	10087538	20 minutes	High pH HIER	Dako Envision FLEX	None	DAB
139	100	ready use	Ventana	E00223	32 min.	CC1	Iview	no	DAB
141	100	Ready to Use	Ventana/Roche	D05144	12 minutes	HIER 30 minutes	ultraView	None	DAB
143	100	no	Confirm ventana	D03841	32 min	CC1 60 min	I view DAB detection kit	endogenous biotin kit	no
145	SP2	1/40	BIOCARE	120513	60 mins	CC1 32 mins	XT OPTIVIEW DAB IHC v4	NO	DAB
146	636	RTU	Dako	10072688	30	Flex TRS High	EnVision Flex Peroxydase	none	DAB
147	16	1:800	Novocastra	1312100	15 mins	Bond ER2 (pH9)	Bond Refine	N/A	DAB
149	PgR636 IR068	RTU	Dako	10088041	20 min	PT Link pH 9 98 C 20 min	Envision Flex Plus	Yes	DAB
150	100	n/a	ventana	0	32min	cc1	ultraview	copper	dab
151	1A6	1:200	NCL	1312116	15MIN	pH6.0/20MIN	POLYMER	NO	DAB
152	100	None	Ventana	54489	60 minutes	CC1	iView	Copper sulfate	DAB
153	100	S/O	Ventana	E00223	24 minutes	CC1 30 minutes	UltraView Dab	Ultra view Copper	DAB
155	100	Predilute	Ventana	E00223	40 min.	CC1	UltraView dab	UV copper	dab
157	IE 2	PRE DILUTED	VENTANA	--	--	CC1 30 MIN	OPTIVIEW BWNCHMARK XT	YES	YES
159	PgR636	prediluted	DAKO	10088041	30'	High	FLEX	none	DAB
160	100	No	Ventana	D09110	8 min	EDTA pH8	Ventana Ultraview	No	DAB
161	PgR636	RTU	DAKO	10088717	20 minutes	High EDTA TRIS buffer	Envision Flex	mouse linker	DAB
162	16	1:80	Leica	6020162	320min	CC1 48 min	OptiView DAB	-	OptiView DAB
163	IE"	Prediluted	Benchmark Ventana	E00223	32 min	HIER	Peroxydase	copper	peroxydase
164	100	predilute	Ventana	E00223	12 minutes	CC1	UltraView	no	DAB
165	100	NIL	VENTANA	D09634	24 MIN	CC1	ULTRAVIEW DAB	ULTRAVIEW	DAB
167	100	Predilute	Ventana (Roche)	D07381	8 mn	CC1 (Roche)	DAB	Copper	DAB
168	PgR 636	RTU	Dako	10068717	20 min.	High pH	Envision Flex	Mouse Linker	DAB
170	PgR636	ready to use	Dako	10087538	20 min	HIER ph high	Envision Flex Dako	no	DAB
173	100	Pre-diluted	Ventana	d09634	16 minutes	CC1	UltraView	Polymer	DAB
175	100	Predilute	Ventana	E000223	32	CC1	UltraView	copper	DAP
177	636	1 : 25	Dako	.	32m	yes	UltraView	yes	DAB
178	100	none	Ventana	na	16 min.	32 min.	UltraView	none	DAB
183	100	PREDILUTE	VENTANA	D09634	32 MIN	CC1	ULTRAVIEW	NONE	DAB
184	PgR636	RTU	Dako	10075666	20	HIER	Envision FLEX+	na	Envision DAB+
186	PR88	1:100	Biogenex	MJ3281213	15'	HIER EDTA PH9	BOND REFINE DETECTION KIT	NONE	DAB
187	IE2	Predilute	Roche	E00223	12	CC1	Optiview	None	DAB
188	16	1/100	Leica Biosystems	Current	30 mins RT	pH 6	Novolink	None	DAB
189	100	pre-dilute	Ventana	??	16 min./37A°	CC1 Standard	UltraView	None	UltraView DAB
190	no change	no change	no change	no change	no change	no change	no change	no change	no change
191	100	RTU	Roche	c04884	16'	CC1	ultraview	none	dab
192	100	READY TO USE	VENTANA	E00223	16 MIN	Ultra CC1 (36 MIN)	Ventana UltraViewDAB	Copper	DAB
194	IE2	PREDILUTE	VENTANA	D09110	20 MINS.	CC1	AVIDIN BIOTIN(IVIEW)	COPPER	DAB
196	100		VENTANA	D09634	8 MINUTES				DAB
198	16	1/400	Vector Laboratories	6020167	30min RT	Citrate pH 6.2	Polymer- MACH 1	Copper Sulphate	DAB
199	16	200	Leica Microsystems	6027462	15 mins	hier (Bond ER-2)	Polymer-Bond Refine	None	DAB
202	1.60e	rtu	leica	26967	15 min	er2 for 30 min	leica refine detection kit	none	dab
207	100	PREDILUTED	VENTANA	D09110	16 MINUTES	CC1-36	ULTRAVIEW	NONE	DAB
208	PgR636	Predilute	Dako	10090679	60 min	Ph 9	Envision Flex	-	DAB flex
209	PgR 636	NA (Pre dilute)	Dako	10088041	20mins	20 mins at 97C in Tris/EDTA buffer and cool down for 20mins to 85C	Polymer	Non	DAB

Table S3. Reported HER2 staining protocols.

Lab ID	Clone	Dilution	Supplier	Ab Lot #	Ab Incubation Time	Ag Retrieval	Detection	Enhancement	Chromogen
101	SP3	1:200	Lab Vision	9103S1202D	32 minutes	CC1 32 minutes	Optiview	Copper	DAB
102	SP3	1/600	THERMO	9103S1305H	30" RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CUS04	DAB+
103	4B5	prediluted	VENTANA	D07696	16'	CC1 36'	Ultraview	Copper	DAB
105	4B5	Neat	Ventana	D02312	32 minutes with heat	CC1 standard	DABMAP	none	DAB
106	4B5	N/A	Ventana	E00842	16 minutes	36 min, 90C, CC1	Ultraview	Copper	DAB
107	4B5	Pre-diluted	Ventana	D09631	8 min @ RT	Ultra CC1 - 36 min	Ultraview DAB	NA	DAB
109	4B5	RTU	VENTANA	E00842	16 minutes	ULTRA CC1-36MIN@95	ULTRAVIEW DAB	COPPER	DAB
111	4B5	Predilute	Ventana	D09631	32min, RT	CC1 - 36 min	2-step multimer	Copper	DAB
112	4B5	RTU	VENTANA	D09098	16 MINUTES	CC1 MILD	ULTRAVIEW	COPPER	DAB
114	SP3	1/200	Thermo	9101S1308F	16mins	CC1 32mins	OptiView	non	DAB
115	4B5	Prediluted	Ventana	E00842	24 min	CC1	IVIEW DAB	COOPER	DAB
116	SP3	1/100	ThermoScientific	1305G	36 min	CC1 40 min	Optiview DAB	no	DAB
119	A0485	1/1000	Dako	--	--	HIER/CC!	uutraview	-	DAB
120	Her2 Protein	prediluted	Dako	20007643	30 min	yes 40 min	Dako Autostainer Link 48	-	Dab
123	4B5	predilute	Ventana/Roche	D09631	32	CC1 for 30 min	ultraview	copper	DAB
124	4B5	none	Ventana	DO9631	12 min	CC1 30 min	IView	none	DAB
125	4B5	predilute	Roche	E00842	16 min	Ventana Ultra CC1 standard	Ventana UltraView	na	DAB
126	SP3	1:400	ThermoFisher	9103S1306I	30min	Steamer	Rabbit EnVision	N/A	DAB
129	A0485	1:600	Dako	86782	15 min	20 min	Bond Refine	no	DAB
133	4B5	predilute	Ventana	D08847	24 minutes	36 minutes CC1	Ultraview Ventana	none	Dab
135	A0485	1:700	Dako	81393	15 min	Bond ER1 20 min	Bond polymerrefined detection	none	DAB
138	A0485	1:600	Dako	92075	30 minutes	Low pH HIER	Dako Envision FLEX	none	DAB
139	4B5	ready use	Ventana	E00035	32 min	CC1	Iview Dab	no	DAB
145	SP3	1/600	CELL MARQUE	1201010C	48 mns	CC1 32 mns	XT OPTIVIEW DAB IHC v4	NO	DAB
147	Polyclonal	1:400	Dako	92075	15 mins	Bond ER1 (pH6)	Bond Refine	N/A	DAB
149	SPC3 Mono R	1:100	ThermoRM 4103-5	91031305	30 min	PT Link pH 9 98 C 20 min	Envision Flex Plus	Yes	DAB
150	sp3	1/100	neomarker	0	32min	cc1	ultraview	copper	dab
151	A0485	1:450	DAKO	92075	15MIN	pH 6.0/20MIN	POLYMER/BOND REFINE	NO	DAB
152	4B5	None	Ventana	54444	60 minutes	CC1	IVIEW	Copper sulfate	DAB
153	4B5	s/o	Ventana	E00842	12 minutes	CC1 30 min	Ultraview Dab	Ultraview Cooper	Dab
155	4B5	predilute	Ventana	D11261	32 min	CC1	Ultraview dab	Uv copper	dab
157	4B 5	predilute	VENTANA	--	--	CC1 30 MIN	OPTIVIEW BENCHMARK XT	YES	YES
160	A0485	1/700	Dako	92075	32 min	EDTA pH8	Ventana Ultraview	No	DAB
161	Rabbit anti-hum	RTU	Dako	20011449	30 minutes	Herceptest epitope retrieval solution	Herceptest visualization reagent	no	Herceptest DAB
162	4B5	RTU	Ventana/Roche	D09098	20 min	CC1 32 min.	UltraView DAB	-	UltraView DAB
164	4B5	predilute	Ventane	D09631	12 minutes	CC1	Ultraview	no	DAB
167	A0485	1/1700	Dako	86782	32 min	CC1 (Roche)	DAB	Copper	DAB
170	ErB-2	ready to use	Dako	20009275	30 min	HIER ph low	Herceptest	no	DAB
175	4B5	predilute	Ventana	D11261	12	CC1	Ultraview	Copper	DAP
181	4B5	pre-diluted	Ventana/Roche	D08847	16 minutes	CC1 for 30 minutes	Ventana Ultraview DAB	Copper Sulfate	DAB
186	POLYCLONAL	1:400	DAKO	20002512	15'	HIER CITRATE PH6	BOND REFINE DETECTION KIT	NONE	DAB
187	4B5	Predilute	Roche	D09631	24	CC1	Optiview	None	DAB
188	CB11	RTU	Leica Biosystems	Current	30 mins	ER1 25 mins	Oracle	None	DAB
189	4B5	pre-dilute	Ventana	??	16min/37Å°	CC1 mild	Ultraview	None	Ultraview DAB
190	no change	no change	no change	no change	no change	no change	no change	no change	no change
191	4B5	RTU	Roche	D08847	16'	CC1	ultraview	none	DAB
194	4B5	PREDILUTE	VENTANA	E00842	12 MINS.	CC1	AVIDIN BIOTIN(IVIEW)	COPPER	DAB
198	4B5	prediluted	Ventana/Roche	D09631	20min	CC1	Ultraview	Copper Sulphate	DAB
199	CB11	RTU	Leica Microsystems	25367	30 min	hier (Bond ER-2)	Polymer-Bond Oracle Kit	None	DAB
202	Her2	rtu	dako	2011449	30 min	40 min at 90C	Hercept kit	none	dab
207	4b5	prediluted	Ventana	E00842	16 MINUTES	CC1-36	ULTRAVIEW	NONE	dab