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Assessors' report for cIQc Run Q3: ER, PR and HER2 (March 2017)

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Assessment performed on July 7, 2017 and July 13, 2017 at Vancouver General Hospital, Vancouver, BC.

OVERVIEW

Run 73 Breast Module (ER, PR, and HER2) consisted of a 40 single-core tissue microarray of breast carcinomas enriched for HER2 3+ cases. The rate of unsatisfactory cores due to core dropout or no tumour present was higher than usual, but a sufficient number of cores still remained. All laboratories were considered to have either optimal or adequate staining. Congratulations to all participants!

ER

Core 40 was noted to have sampling variability, with staining in only a few tumour cells. Cores 8, 23 and 30 were weakly positive with only a few tumour cells (i.e. Allred 3-5), but provided a good measure to distinguish optimal from adequate staining by participants. A lab was considered to have optimal staining if results were completely concordant with the reference or at most one of the weakly staining tumour cores was negative. If two or three of the weakly staining cores were negative, staining was considered adequate.

Participant-specific feedback is summarized below:

Table with 3 columns: Lab ID, IHC Status*, Comments. Rows 101-147.

*based on cIQc assessment

Table with 3 columns: Lab ID, IHC Status*, Comments. Rows 148-233.

*based on cIQc assessment



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PR

As there is no accepted "gold standard" for comparison, the consensus of results from participants remains the cIQc approach. Interpretation of PR results must also be done in the context of ER. For instance, variable PR positivity in an ER-positive core is clinically irrelevant. However, variable PR positivity in an ER-negative core may indicate a technical problem given the rarity of ER-/PR+ cases. Sampling variability was evident in several cores; notably Cores 7, 25, 26 and 35, which had focal staining in only a few tumour cells. All labs exhibited either optimal or adequate staining. Participant-specific feedback is summarized below:

Table with 3 columns: Lab ID, IHC Status*, Comments. Lists lab results for labs 101-147.

*based on cIQc assessment

Table with 3 columns: Lab ID, IHC Status*, Comments. Lists lab results for labs 149-233.

*based on cIQc assessment

Large grid table with 40 rows (Cores) and 23 columns (Labs). Each cell contains a letter (U, N, P) representing staining results.



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HER2

No false positive or negative results were observed, with the exception of Lab 101 which appeared to have had a technical issue with their slide. Core 23 was previously noted to be a case with amplification of HER2 but relatively low-level protein expression, leading to variable immunohistochemical results that were excluded from all analyses. At this time, 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. Sampling variability was noted in Core 14. Participant-specific feedback is summarized below:

Lab ID	IHC Status*	Comments
101	--	Technical issue occurred during staining
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	Slightly weak
109	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
120	--	Slide not available for assessment
123	--	Slide not available for assessment
125	Optimal	
126	Optimal	
127	Optimal	
129	Optimal	
133	Optimal	
138	Optimal	
147	Optimal	

Lab ID	IHC Status*	Comments
149	Optimal	Slightly weak
151	Adequate	
175	Optimal	
181	Optimal	
186	Optimal	
187	Optimal	
189	--	Slide not available for assessment
190	Optimal	
194	Optimal	
198	Optimal	
199	--	Slide not available for assessment
202	Optimal	
207	Optimal	
217	Optimal	
221	Optimal	Strong counterstain
230	Optimal	
233	Optimal	Nice staining

*based on cIQc assessment

*based on cIQc assessment

Lab/Core	101	102	103	106	107	109	111	112	114	120	123	125	126	127	129	133	138	147	149	151	175	181	186	187	189	190	194	198	199	202	207	217	221	230	233	FISH					
1	U	U	U	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	U	U	3	3	3	3	U	U	U	3	3	3	3	3	3	3	3	U	Amplified			
2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	U	U	3	3	3	3	U	U	U	3	3	3	3	3	3	3	3	U	Amplified			
3	N	1	2	1	N	1	1	1	N	1	N	N	1	1	N	1	1	1	N	1	1	2	2	N	1	N	1	N	N	1	1	2	1	N	N	U	Non-amplified				
4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	U	U	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	Amplified			
5	3	3	3	3	3	3	3	U	3	3	3	3	3	3	3	3	3	3	U	U	U	3	3	3	3	U	3	3	3	3	3	3	3	3	3	3	U	Amplified			
6	N	1	1	N	N	N	N	N	N	N	N	N	1	N	N	N	N	1	N	1	N	1	N	1	N	N	1	N	N	N	N	N	N	N	N	U	Non-amplified				
7	U	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	U	U	U	U	U	U	U	U	1	N	N	U	1	N	N	U	--				
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	U	U	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	Amplified			
9	N	1	1	N	N	1	N	N	N	2	1	1	1	1	1	1	1	1	2	N	1	N	2	1	N	1	1	1	1	1	1	1	1	1	1	1	U	Non-amplified			
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	N	N	N	N	N	N	N	U	Non-amplified			
11	1	U	3	U	3	3	2	3	3	3	3	3	3	3	3	3	2	3	3	2	3	3	2	3	2	2	3	3	3	3	3	3	3	3	3	U	Amplified				
12	U	U	U	U	3	3	U	3	U	3	U	3	U	3	U	3	U	3	U	U	U	3	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Amplified		
13	U	U	U	U	N	N	U	1	U	1	N	U	1	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Non-amplified		
14	3	U	3	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	3	3	3	U	U	U	2	U	3	U	U	3	2	3	3	U	U	U	U	--			
15	U	N	N	N	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	U	Non-amplified		
16	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	3	3	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Amplified		
17	N	U	2	U	N	1	U	1	U	U	1	U	U	1	U	U	1	N	1	U	1	N	1	U	1	N	1	U	U	1	2	1	U	U	U	U	U	U	Non-amplified		
18	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	1	N	N	N	1	N	N	N	1	N	N	N	N	1	N	N	N	N	N	N	N	U	--		
19	U	U	U	U	N	U	U	N	U	1	N	U	U	1	N	U	U	U	U	N	N	N	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Non-amplified	
20	1	1	1	N	N	N	N	N	N	N	N	1	N	1	1	1	2	N	U	N	1	1	N	1	2	1	N	1	1	1	2	1	N	1	1	2	N	U	--		
21	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Non-amplified		
22	U	U	U	U	N	N	U	N	U	N	U	N	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Non-amplified	
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25	U	U	N	U	1	1	U	2	U	N	1	U	U	1	U	U	U	U	U	N	N	U	U	N	U	N	U	N	U	U	U	U	U	U	U	U	U	U	--		
26	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	U	U	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	Amplified		
27	U	N	U	N	U	N	N	N	N	N	N	N	N	N	N	N	N	U	U	U	N	N	N	N	U	U	N	U	U	U	U	U	U	U	U	U	U	U	U	Non-amplified	
28	U	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	--	
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30	3	U	3	U	2	3	U	3	3	3	3	3	3	3	3	3	3	U	U	3	3	3	3	3	3	U	U	2	U	2	U	U	U	U	U	U	U	U	Amplified		
31	U	U	U	U	3	3	U	3	U	1	2	U	U	3	3	U	U	U	U	2	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Amplified	
32	1	2	1	N	N	N	N	N	1	N	N	1	1	2	N	1	2	N	1	2	N	1	N	N	1	1	N	1	1	N	2	1	N	1	N	1	U	Non-amplified			
33	U	1	U	1	U	1	N	1	N	1	1	1	1	U	1	N	1	1	U	U	1	2	1	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	--	
34	U	U	U	U	N	N	N	N	N	N	N	1	N	N	N	N	N	U	U	1	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Non-amplified	
35	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	1	N	N	N	N	N	N	N	N	U	Non-amplified	
36	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	U	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	Amplified		
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	N	N	N	N	N	N	N	N	N	N	N	N	U	Non-amplified	
38	1	1	2	1	N	1	N	1	1	1	1	1	1	1	1	2	2	1	2	N	1	1	2	1	N	1	1	1	1	1	1	1	1	1	1	1	1	U	Non-amplified		
39	U	U	U	U	U	U	U	1	U	U	N	U	2	1	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	--
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	N	N	N	N	N	N	N	N	N	U	Non-amplified	



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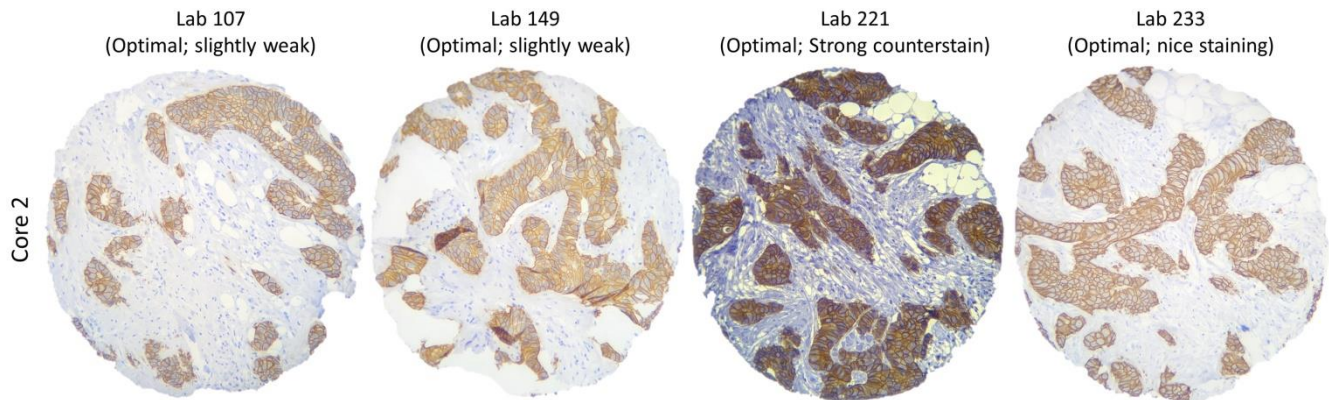


Figure 2. Representative images of qualitative variability of HER2 staining seen in Core 4 (HER2-amplified).

Supplementary Tables 1-3 summarizing staining protocols and Supplementary Tables 4-6 summarizing descriptive statistics can be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Your participation in cIQc through the INSPQ is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Table S1. Reported ER staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 min	SP1	1:50	THERMO/LAB VISION	9101S1604A	32min	Optiview	NN	N	DAB
102	DAKO PT - HIGH PH	20	EP1	1:50	DAKO	10114095	30" RT	DAKO ENVISION FLEX	NO	CUSO4	DAB+
103	CC1	64 MINS	SP1	PRE	VENTANA	G03194	16 MINS	ULTRA VIEW	N	Y	DAB
106	CC1	64	SP1	predilute	Roche	G08481	32	Optiview	no	no	DAB
107	Ultra cc1	36	SP1	Pre-diluted	Ventana	G05134	16	Ultraview DAB	N	Y	DAB
109	HIER high pH	64 MIN	SP1	RTU	ROCHE/VENTANA	G06054	64 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	36	SP1	predilute	Ventana	G06054	32	ultraview	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	SP1	1:200	ThermoFisher	9101S1604E	15 minutes @ RT	BOND polymer refine	no	no	DAB
114	CC1	32	SP1	1/50	Thermo Fisher	9101S1501J	16	Optiview	N	Y	DAB
120	waterbath (TRS High)	20	EP1	ready to use	Dako by Agilent	10110172	20	Envision Flex	N	N	DAB
126	Steam-Citrate pH 6.0	45 min	SP1	1:200	Thermo Scientific	9101s1604E	30	Envision Plus	N	N	Dab Plus
127	AUTOMATED (CC1 ON BENCHMARK ULTRA)	36	SP1	PREDILUTE	VENTANA	G00585	32	ULTRAVIEW DAB DETECTION KIT	N	N	DAB
128	CC1	64 min	SP1	Pre-dilute	Ventana	G06054	16 min	Ultraview	No	No	DAB
129	ER 2- high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1604K	15	Bond Refine Detection Kit	N	N	DAB
132	Flex TRS High	20	EP1	RTU	Dako/Agilent	10120130	20	Flex 20	N	N	DAB
133	heir	36	SP1	pre dilute	roche	F09522	32	ultraview	n	n	dab
134	HIER - CC1	30	SP1	RTU	VENTANA/ROCHE	G00585	8	ULTRAVIEW	N	N	DAB
138	HIER	20	EP1	RTU	Dako	10117622	20	Dako Envision Flex	N	N	DAB
141	HIER-CC1	30	SP1	RTU	VENTANA/ROCHE	G00585	8	ULTRAVIEW	N	N	DAB
144	CC1	24 min.	SP1	1:50	ThermoScientific	1604H	16 min.	OptiView	No	Yes, copper	DAB
147	HIER-PH 9	20	SP1	1:100	THERMO	910151604D	15	REFINE POLYMER	N	N	DAB
148	CC1	36 min	SP1	RTU	Ventana	G06054	12 min	Ultraview	N	N	DAB
149	PT Link high pH	20 min at 97 C	EP1	RTU	Dako	10120130	20	EnVision Flex	No	No	DAB
151	BUFFER PH 9.0	20 MIN	SP1	1:25	THERMO FISHER	9101S1604K	15 MIN	BOND REFINE	N	N	DAB
175	hier	36 min	SP1	predilute	Roche	G06054	32 mins	polymer	N	Y-copper	DAB
178	HIER	32	SP1	none	Ventana	G04150	16	Ultraview	N	N	DAB
183	Ultra CC1	36	SP1	RTU	Ventana/Roche	G06054	32	Ultraview DAB	N	N	DAB
186	HIER	20	SP1	1:50	ThermoScientific	9101S1604E	15	POLYMER	N	N	DAB
187	CC1	16	SP1	Predilute	Roche	G08481	8	Optiview	N	N	DAB
189	CC1	64	SP1	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	SP1 rabbit	n/a	VENTANA	G06054	32	IVIEW	N	N	DAB
192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	G08481	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	SP1	PREDILUTE	VENTANA	G03194	12	IVIEW	Y	Y	DAB
198	Envision Flex Tris high pH	30 min	EP1	Prediluted	Dako/Agilent	10120130	17 min	Envision Flex/ HRP	N	N	DAB
199	HIER (ER-1)	20	6F11	RTU	LEICA	48652	15	Bond Refine (Polymer)	N	N	DAB
202	HIER citrate pH 1	20	6f11	1/150	leica	6044415	15	Leica Refine detection kit	no	no	dab
207	CC1-ON LINE	36 minutes	SP1	prediluted	VENTANA	G05134	16 minutes	ultraview	N	Y	DAB
209	HIER	20 mins at 97C and then 20mins cooling down to 85C	EP1	N/A (Predilute)	Dako	10120130	20mins	Envision plus	N	N	DAB
221	pH6 Citrate Buffer	20	SP1	1:100	Cell Marque	1603507B	30	Rabbit EnVision	N	N	DAB
230	HIER	64	SP1	NONE	VENTANA	G08481	32	IVIEW	N	N	DAB
232	On line retrieval	20 mins	6F11	predilute	Leica	46330	15 mins	Refine Detection	N	N	DAB
233	CC1	36	SP1	NA	Roche	G08481	16	Ultraview Universal DAB Detection Kit	N	N	DAB

Table S2. Reported PR staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 min	16	1:100	Novocastra	6015355	32min	Optiview	N	N	DAB
102	DAKO PT - HIGH PH	20	16	1:250	NOVOCASTRA	6044509	30	DAKO ENVISION FLEX	NO	CUSO4	DAB+
103	CC1	64 MINS	1E2	PRE	VENTANA	G05674	16 MINS	ULTRA VIEW	N	Y	DAB
106	CC1	64	PgR1294	1:220	Dako	10117646	32	Optiview	no	no	DAB
107	Ultra cc1	64	PgR1294	1:50	Dako	10121367	32	Ultraview DAB	Y	Y	DAB
109	HIER high pH	36 MIN	1294	1/50	DAKO	10114753	16 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	48	16	80	Leica	6041139	32	optiview	N	Y	DAB
112	BOND ER2 pH 9.0	12 minutes	16	RTU	LEICA	48654	15 minutes	BOND polymer refine	no	no	DAB
114	CC1	32	16	1/25	Novocastra	6044509	16	Optiview	N	Y	DAB
120	waterbath (TRS High)	20	PgR 636	ready to use	Dako by Agilent	10109504	20	Envision Flex	Y	N	DAB
126	Steam-Citrate pH6.0	45	630	1:500	Dako	10110361	30	Envision Plus	N	N	Dab Plus
127	AUTOMATED (CC1 ON BENCHMARK ULTRA)	36	1E2	PREDILUTE	VENTANA	F09520	8	ULTRAVIEW DAB DETECTION KIT	N	N	DAB
128	CC1	64 min	1E2	Pre-dilute	Ventana	G06454	16 min	Ultraview	No	No	DAB
129	ER 2- high pH retrieval	20	16	1:400	Novocastra	6027295	15	Bond Refine Detection Kit	N	N	DAB
132	Flex TRS High	20	16	1:200	Dako/Agilent	6041139	30	Flex 20	N	N	DAB
133	heir	64	16	1/25	Leica	6041139	60	Ultraview	n	n	dab
134	HIER - CC1	30	1E2	RTU	VENTANA/ROCHE	G04962	12	ULTRAVIEW	N	N	DAB
138	HIER	20	636	RTU	Dako	10117079	20	Dako Envision Flex	Y	Y	DAB
141	HIER-CC1	30	1E2	RTU	VENTANA/ROCHE	G04962	12	ULTRAVIEW	N	N	DAB
147	HIER-PH 9	20	16	1:800	NCL	6027295	15	REFINE POLYMER	N	N	DAB
149	PT Link high pH	20 min at 97 C	PgR636	RTU	Dako	10117079	20	EnVision Flex	Yes	No	DAB
151	BUFFER PH 6.0	20 MIN	1A6	1:200	ncl	NCL	15 min	BOND REFINE	N	N	DAB
178	HIER	32	1E2	none	Ventana	G05131	16	Ultraview	N	N	DAB
183	Ultra CC1	36	1E2	RTU	Ventana/Roche	F10358	32	Ultraview DAB	N	N	DAB
186	HIER	20	PR88	1:100	BIOGENEX	MU3280415	15	POLYMER	N	N	DAB
187	CC1	64	1E2	Predilute	Roche	G06454	12	Ultraview	N	N	DAB
189	CC1	64	1E2	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	clone 16	1:50	NOVACASTRA	6027462	32	IVIEW	N	N	DAB
192	Ultra CC1	36 minutes	1E2	Ready to use	Ventana/Roche	G06454	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	1E2	PREDILUTE	VENTANA	G04962	20	IVIEW	N	Y	DAB
198	Envision Flex Tris high pH	30 min	1294	1/100	Dako/Agilent	10117647	20 min	Envision Flex/ HRP	N	N	DAB
199	HIER (ER-2)	20	16	200	LEICA	6046807	15	Bond Refine (Polymer)	N	N	DAB
202	HIER citrate pH 9.5	30	1.6	rtu	Leica	47688	15	Leica Refine detection kit	no	no	DAB
207	CC1-ON LINE	36 minutes	1E2	prediluted	VENTANA	G05674	16 minutes	ultraview	N	Y	DAB
209	HIER	20mins at 97C and 20mins cooling down to 85C	PgR636	N/A (pre dilute)	Dako	10117079	20min	Envision Plus	Y	N	DAB
221	pH6 Citrate Buffer	20	1A6	1:200	Leica	3054DKP	30	Mouse EnVision	N	N	DAB
230	HIER	32	1E2	NONE	VENTANA	G08195	32	IVIEW	N	N	DAB
232	On line retrieval	20 mins	16	predilute	Leica	42131	15 mins	Refine Detection	N	N	DAB
233	CC1	48	16	1/100	Leica	6041139	32	Optiview DAB	N	N	DAB

Table S3. Reported HER2 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	ICC	32 min	SP3	1:200	THERMO/LAB VISION	9103S15090	32min	Optiview	N	N	DAB
102	DAKO PT - HIGH PH	20	SP3	1:75	THERMO FISHER	9103S1606F	30' RT	DAKO ENVISION FLEX	NO	CUSO4	DAB+
103	CC1	36 MINS	4B5	PRE	VENTANA	G03109	16 MINS	ULTRA VIEW	N	Y	DAB
106	CC1	36	4B5	predilute	Roche	G08338	16	Ultraview	no	no	DAB
107	Ultra cc1	36	4B5	Pre-diluted	Ventana	G04794	12	Ultraview DAB	N	Y	DAB
109	HIER high pH	36 MIN	4B5	RTU	ROCHE/VENTANA	G08338	16 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	36	4B5	pre-dilute	Ventana	G04794	32	Ultraview	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	4B5	1:4 ratio of the RTU	Ventana/Roche	G00135	15 minutes @ RT	BOND polymer refine	no	no	DAB
114	CC1	32	SP3	1/200	Thermo Fisher	9103S1509D	32	Optiview	N	Y	DAB
120	waterbath (HercepTest)	40	HER2 protein	ready to use	Dako by Agilent	20038535	30	HercepTest	N	N	DAB
126	CC1 (Ventana)	56	4B5	Prediluted	Ventana	G10339	20	UltraView (Ventana)	N	N	Ultraview DAB detection-Biotin Free
127	AUTOMATED (CC1 ON BENCHMARK ULTRA)	36	4B5	PREDILUTE	VENTANA	G06737	24	ULTRAVIEW DAB DETECTION KIT	N	N	DAB
129	ER 2- high pH retrieval	20	SP3	1:100	Thermo Scientific	RL2310171	15	Bond Refine Detection Kit	N	N	DAB
133	heir	36	4b5	pre dilute	roche	G04794	24	Ultraview	n	n	dab
138	HIER	40	HercepTest (A0485)	RTU	Dako	20038535	30	Dako Envision Flex	N	N	DAB
147	HIER_PH9	20	SP3	1:75	THERMO	RH2258R1	15	REFINE POLYMER	N	N	DAB
149	PT Link low pH	20 min at 97 C	A0485 PolyR	1:500	Dako	20027850	20	EnVision Flex	Yes	No	DAB
151	BUFFER PH 9.0	20 MIN	SP3	1:75	THERMO	RA2131961	15 MIN	DOND REFINE	N	N	DAB
175	hier	32 mins	4B5	predilute	Roche	G06737	16 mins	polymer	N	Y-copper	DAB
181	CC1 on board	30 minutes	4B5	pre-diluted	Ventana/Roche	G04794	16 minutes	Ventana Ultraview DAB	no	yes	DAB
186	HIER	20	POLYCLONAL	1:400	DAKO	20023582	15	POLYMER	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	G04794	24	Optiview	N	N	DAB
189	CC1	32	4B5	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	SP3 rabbit	1:50	Thermofisher	9103S1509A	40	iVIEW	Y	N	DAB
194	CC1	30	4B5	PREDILUTE	VENTANA	G09203	12	iVIEW	N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Roche/Ventana	G04794	32 min	Ultraview	N	Y	DAB3
199	HIER (ER-2)	20	SP3	300	CELL MARQUE	1602506A	15	Bond Refine (Polymer)	N	N	DAB
202	citrate buffer	40 min	Her2	RTU DAKO	DAKO	20040935	30	Herceptest kit	no	no	dab
207	cc1- on line	36 minutes	4B5	prediluted	VENTANA	G09203	16 miutes	ultraview	N	Y	DAB
221	Dako Visualization Solution	40	Dako Hercep Test	NEAT	Dako	20038525	30	Dako Kit	N	N	DAB
230	HIER	30	4B5	NONE	VENTANA	G08338	16	iVIEW	N	N	DAB
233	CC1	36	4B5	NA	Roche	G09203	16	Ultraview Universal DAB Detection Kit	N	N	DAB

Table S4. Descriptive statistics for ER based on cIQc assessment. Core 40 was excluded from analysis due to sampling variability.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	39	69.23	25	24/25 (96%)	0.95	1	0.88
102	39	56.41	21	20/21 (95%)	0.94	1	0.88
103	39	66.67	24	23/24 (96%)	0.94	1	0.9
106	39	69.23	26	26/26 (100%)	1	1	1
107	39	74.36	28	27/28 (96%)	0.95	1	0.9
109	39	58.97	22	21/22 (95%)	0.94	1	0.88
111	39	66.67	25	25/25 (100%)	1	1	1
112	39	89.74	34	33/34 (97%)	0.96	1	0.92
114	39	89.74	34	34/34 (100%)	1	1	1
120	39	69.23	25	24/25 (96%)	0.94	1	0.91
123	39	89.74	34	34/34 (100%)	1	1	1
125	39	89.74	34	34/34 (100%)	1	1	1
126	39	56.41	21	20/21 (95%)	0.94	1	0.88
127	39	89.74	34	34/34 (100%)	1	1	1
128	39	94.87	35	35/35 (100%)	1	1	1
129	39	89.74	34	34/34 (100%)	1	1	1
132	39	74.36	28	27/28 (96%)	0.95	1	0.91
133	39	79.49	30	30/30 (100%)	1	1	1
134	39	61.54	22	22/22 (100%)	1	1	1
138	39	61.54	22	20/22 (91%)	0.89	1	0.7
141	39	66.67	24	23/24 (96%)	0.95	1	0.88
144	39	64.1	23	22/23 (96%)	0.95	1	0.86
147	39	66.67	24	23/24 (96%)	1	0.8	0.86
148	39	61.54	22	22/22 (100%)	1	1	1
149	39	64.1	23	22/23 (96%)	0.95	1	0.86
151	39	61.54	22	22/22 (100%)	1	1	1
175	39	69.23	26	26/26 (100%)	1	1	1
178	39	66.67	24	24/24 (100%)	1	1	1
183	39	71.79	26	25/26 (96%)	0.95	1	0.9
186	39	76.92	28	27/28 (96%)	0.95	1	0.91
187	39	69.23	25	24/25 (96%)	0.95	1	0.88
189	39	69.23	25	25/25 (100%)	1	1	1
190	39	64.1	23	23/23 (100%)	1	1	1
192	39	94.87	34	34/34 (100%)	1	1	1
194	39	51.28	19	18/19 (95%)	0.93	1	0.85
196	39	66.67	24	23/24 (96%)	0.95	1	0.86
198	39	58.97	21	21/21 (100%)	1	1	1
199	39	66.67	24	23/24 (96%)	0.95	1	0.88
202	39	61.54	22	22/22 (100%)	1	1	1
207	39	64.1	23	22/23 (96%)	0.95	1	0.86
209	39	64.1	23	22/23 (96%)	0.95	1	0.86
217	39	51.28	19	18/19 (95%)	0.93	1	0.85
221	39	56.41	20	19/20 (95%)	0.94	1	0.86
230	39	53.85	20	18/20 (90%)	0.88	1	0.74
232	39	66.67	24	22/24 (92%)	0.89	1	0.8
233	39	61.54	22	21/22 (95%)	0.94	1	0.89

Table S5. Descriptive statistics for PR based on cIQc assessment. Cores 7, 25, 26 and 35 were excluded from analysis due sampling variability.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	36	55.56	20	20/20 (100%)	1	1	1
102	36	55.56	20	19/20 (95%)	0.89	1	0.9
103	36	80.56	28	26/28 (93%)	1	0.87	0.86
106	36	61.11	21	21/21 (100%)	1	1	1
107	36	86.11	30	30/30 (100%)	1	1	1
109	36	66.67	24	24/24 (100%)	1	1	1
111	36	66.67	24	24/24 (100%)	1	1	1
112	36	91.67	31	29/31 (94%)	0.88	1	0.87
114	36	66.67	24	24/24 (100%)	1	1	1
120	36	66.67	23	22/23 (96%)	0.9	1	0.91
123	36	88.89	31	31/31 (100%)	1	1	1
125	36	86.11	30	30/30 (100%)	1	1	1
126	36	58.33	21	21/21 (100%)	1	1	1
127	36	88.89	31	31/31 (100%)	1	1	1
128	36	94.44	32	32/32 (100%)	1	1	1
129	36	80.56	28	28/28 (100%)	1	1	1
132	36	75	27	27/27 (100%)	1	1	1
133	36	58.33	20	20/20 (100%)	1	1	1
134	36	63.89	22	20/22 (91%)	1	0.8	0.81
138	36	63.89	23	23/23 (100%)	1	1	1
141	36	63.89	23	21/23 (91%)	1	0.83	0.83
147	36	69.44	25	25/25 (100%)	1	1	1
149	36	61.11	21	21/21 (100%)	1	1	1
151	36	72.22	26	26/26 (100%)	1	1	1
175	36	75	27	23/27 (85%)	1	0.69	0.7
178	36	77.78	26	25/26 (96%)	0.92	1	0.92
183	36	75	26	26/26 (100%)	1	1	1
186	36	80.56	28	28/28 (100%)	1	1	1
187	36	75	26	26/26 (100%)	1	1	1
189	36	66.67	23	23/23 (100%)	1	1	1
190	36	58.33	20	19/20 (95%)	0.9	1	0.9
192	36	69.44	23	20/23 (87%)	0.9	0.85	0.74
194	36	50	18	17/18 (94%)	1	0.9	0.89
196	36	58.33	20	20/20 (100%)	1	1	1
198	36	61.11	21	21/21 (100%)	1	1	1
199	36	61.11	20	20/20 (100%)	1	1	1
202	36	63.89	22	22/22 (100%)	1	1	1
207	36	63.89	22	22/22 (100%)	1	1	1
209	36	63.89	22	22/22 (100%)	1	1	1
217	36	61.11	21	19/21 (90%)	1	0.82	0.81
221	36	63.89	21	21/21 (100%)	1	1	1
230	36	50	18	17/18 (94%)	1	0.9	0.89
232	36	61.11	21	21/21 (100%)	1	1	1
233	36	61.11	21	21/21 (100%)	1	1	1

Table S6. Descriptive statistics for HER2 based on cIQc assessment. Cores 14 and 23 were excluded from analysis due to the described variability of immunohistochemical staining noted above.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
102	38	57.89	22	22/22 (100%)	1	1	1
103	38	65.79	25	25/25 (100%)	1	1	1
106	38	60.53	23	23/23 (100%)	1	1	1
107	38	84.21	32	32/32 (100%)	1	1	1
109	38	86.84	33	33/33 (100%)	1	1	1
111	38	68.42	26	26/26 (100%)	1	1	1
112	38	89.47	34	34/34 (100%)	1	1	1
114	38	71.05	27	27/27 (100%)	1	1	1
120	38	78.95	30	30/30 (100%)	1	1	1
123	38	92.11	35	35/35 (100%)	1	1	1
125	38	63.16	24	24/24 (100%)	1	1	1
126	38	60.53	23	23/23 (100%)	1	1	1
127	38	92.11	35	35/35 (100%)	1	1	1
129	38	81.58	31	31/31 (100%)	1	1	1
133	38	71.05	27	27/27 (100%)	1	1	1
138	38	65.79	25	25/25 (100%)	1	1	1
147	38	57.89	22	22/22 (100%)	1	1	1
149	38	57.89	22	22/22 (100%)	1	1	1
151	38	55.26	21	21/21 (100%)	1	1	1
175	38	78.95	30	30/30 (100%)	1	1	1
181	38	76.32	29	29/29 (100%)	1	1	1
186	38	76.32	29	29/29 (100%)	1	1	1
187	38	63.16	24	24/24 (100%)	1	1	1
189	38	68.42	26	26/26 (100%)	1	1	1
190	38	63.16	24	24/24 (100%)	1	1	1
194	38	50	19	19/19 (100%)	1	1	1
198	38	73.68	28	28/28 (100%)	1	1	1
199	38	60.53	23	23/23 (100%)	1	1	1
202	38	63.16	24	24/24 (100%)	1	1	1
207	38	57.89	22	22/22 (100%)	1	1	1
217	38	55.26	21	21/21 (100%)	1	1	1
221	38	52.63	20	20/20 (100%)	1	1	1
230	38	50	19	19/19 (100%)	1	1	1
233	38	55.26	21	21/21 (100%)	1	1	1