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Assessors' report for cIQc Run 87: Breast Module (ER, PR, and HER2)

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Assessment performed on June 26, 2018 at Vancouver General Hospital, Vancouver, BC

OVERVIEW

The Run 87 Breast Module (ER, PR, and HER2) consisted of a 46 single-core tissue microarray of breast carcinomas enriched for 2+ HER2 cases from 2009 in order to get a glimpse of the evolution of HER2 staining since then. A 3-core HER2 cell line control (courtesy of StatLab) was also included on most participants' HER2 slide, providing a reference standard for further comparison of IHC staining across laboratories. Based on the self-assessment results of ER, PR and HER2 staining it was anticipated that the overall results would be good and this was correct, with the exception of a handful of labs with what the assessment team considered to have very weak HER2 staining compared to the other laboratories.

ER

Core 22 was a low-grade carcinoma with possible apocrine morphology and variable ER expression (possibly related to variable fixation) such that laboratories with potentially more sensitive staining had weak but unequivocally positive staining. Core 42 had relatively few tumour cells present, leading to some heterogeneity of staining results across laboratories. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Adequate	
102	Optimal	
103	Optimal	
106	Optimal	Dark counterstain
107	Optimal	Nice staining
109	Optimal	
111	Optimal	Slightly weak
112	Optimal	
114	Optimal	
120	Optimal	
123	Optimal	
125	Optimal	
127	Optimal	Nice staining
128	Adequate	
129	Adequate	
132	Optimal	
133	Adequate	
134	Adequate	
138	Optimal	
141	Adequate	Slightly weak
144	Optimal	
147	Optimal	

Lab ID	IHC Status*	Comments
148	Optimal	
149	Adequate	Unusual membranous staining observed in some cores; slight blush
151	Optimal	
175	Optimal	Slightly weak
178	Adequate	Strong counterstain
183	Optimal	
186	Optimal	
187	Optimal	
188	--	Slide not available for assessment
189	Adequate	
190	Optimal	
192	Optimal	
194	--	Slide not available for assessment
196	Optimal	
198	Adequate	
199	Optimal	
202	Adequate	False positive in Core 9
207	Optimal	
209	Optimal	
221	Adequate	
230	Optimal	
233	Optimal	

*Based on cIQc assessor consensus

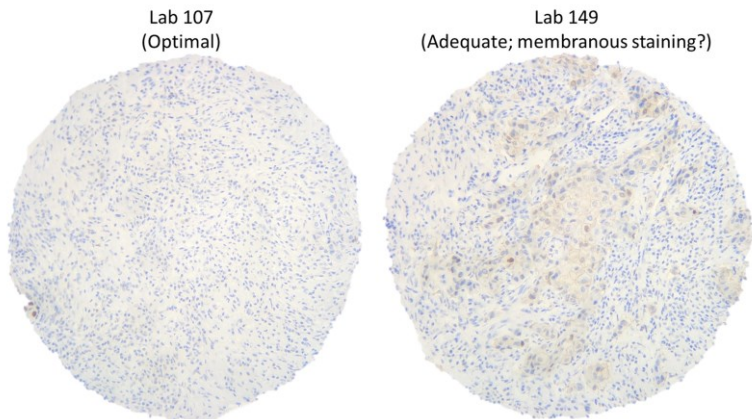


Figure 1. Representative image of the unusual ER membranous staining seen in some cores from Lab 149 compared to the same core in Lab 107.



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HER2

Despite the challenging nature of HER2 cases included in this tissue microarray, there were no false positive results observed on any slides that were submitted in time for review at the assessment meeting. While very weak staining was noted in a handful of laboratories, the majority of participants had optimal staining. Participant-specific feedback is summarized below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	Nice staining
109	Optimal	
111	Optimal	Nice staining
112	Optimal	
114	Optimal	
120	Optimal	Slightly weak
123	Optimal	
125	Optimal	
127	Optimal	
129	Sub-optimal	Very weak staining; especially evident in the HER2 cell line control
133	Optimal	
138	Optimal	
147	Optimal	
149	Optimal	

Lab ID	IHC Status*	Comments
151	Optimal	Nice staining
175	Optimal	
181	Optimal	
186	Optimal	
187	Adequate	Weak staining compared to other labs
188	--	Slide not available for assessment
189	Failed	Extremely weak staining
190	Adequate	Slight background on some cores
194	--	Slide not available for assessment
198	Optimal	
199	Optimal	
202	Optimal	Nice staining
207	Optimal	
221	Optimal	Dark counterstain
230	Optimal	Slightly weak
233	--	Slide not available for assessment
237	Sub-optimal	Very weak staining; especially evident in the HER2 cell line control

*Based on CIQC assessor consensus

Across most laboratories minor variability of staining was noted in the StatLab HER2 cell line controls included on the slide with the tissue microarray. However, for Labs 129 and 237 (sub-optimal IHC status) the very weak intensity staining was immediately evident upon review of this cell line control (Figure 2). Unfortunately, no cell line control was available for Lab 189.

Lab/ Core	101	102	103	106	107	109	111	112	120	125	127	129	133	138	147	149	181	186	187	188	194	198	199	202	207	221	230	237	
1	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	
2	2	2	2	2	2	2	2	2	2	2	3	1	2	2	2	3	2	2	2	2	2	2	2	3	3	2	2	N	
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N

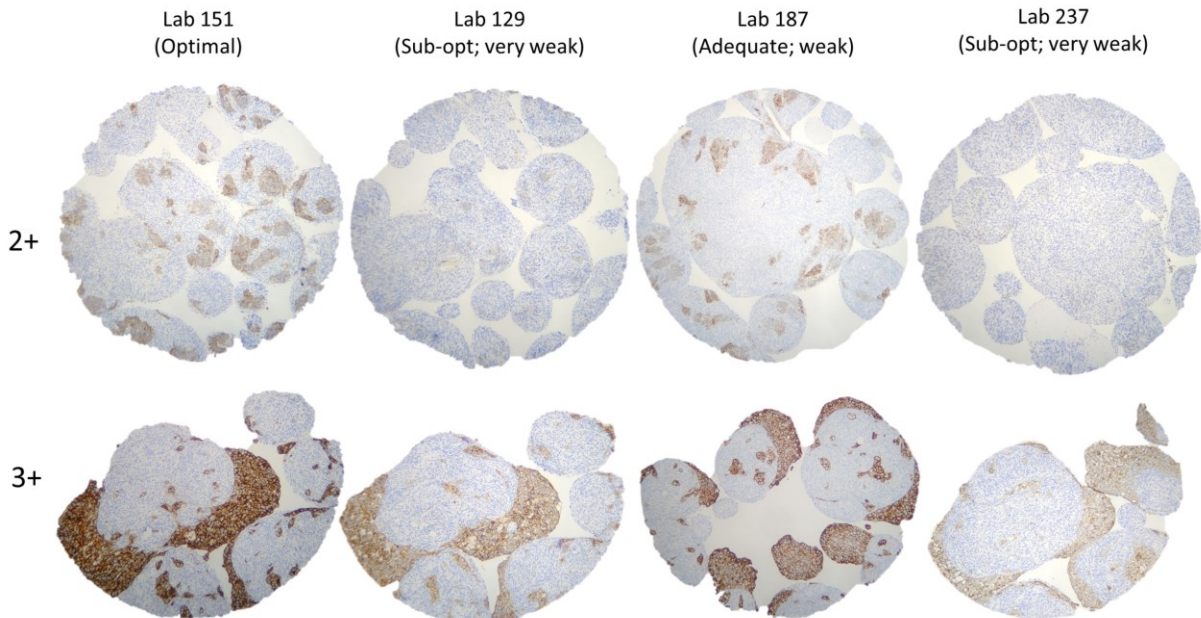


Figure 2. Images of the 2+ and 3+ StatLab HER2 cell line controls in a laboratory with optimal, sub-optimal and adequate staining.



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Lab 151
(Optimal)

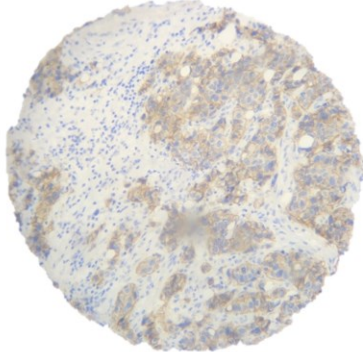


Figure 3. Representative images of a core considered 2+ for most participants. However, Labs 187, 189 and 237 were considered "1+", "N" and "N" respectively.

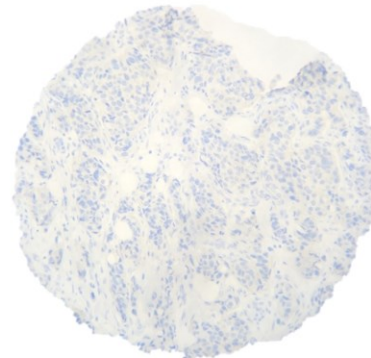
Lab 129
(Sub-opt; very weak)



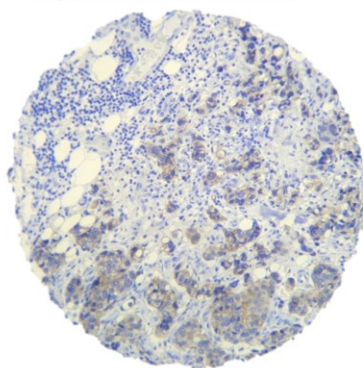
Lab 187
(Adequate; weak)



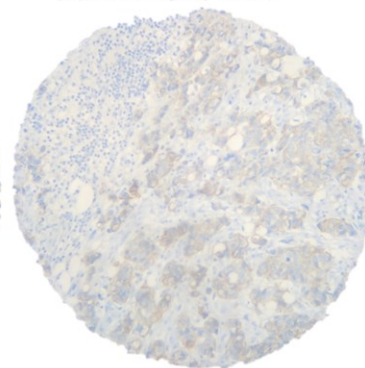
Lab 189
(Failed; extremely weak)



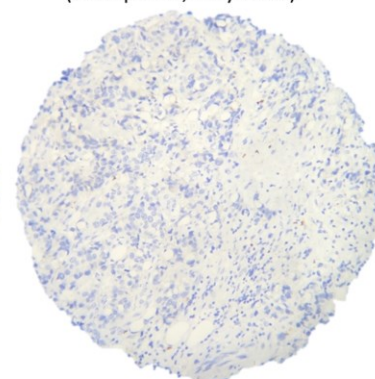
Lab 221
(Optimal; dark counterstain)



Lab 230
(Optimal; slightly weak)



Lab 237
(Sub-optimal; very weak)



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 in mind. Your 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.

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	EnV FlexTRS, High PH	30 min	ER- SP1	1:50	THERMO SCIENTIFIC	9101S1711E	30 min	DAKO Envision Flex	N	N	DAB
102	DAKO PT - HIGH PH	20	EP1	1:50	DAKO	10126585	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
106	CC1	64	SP1	predilute	Roche	Y25308	32	Optiview	no	yes	DAB
107	Ultra cc1	36	SP1	Pre-diluted	Ventana	Y24472	16	Ultraview DAB	N	Y	DAB
109	HIER high pH -CC1	64 MIN	SP1	RTU	ROCHE	E03296	32 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	36	SP1	PREDILUTE	VENTANA	E03296	30	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 min	SP1	1:200	ThermoFisher	9101S1604H	15 min at RT	BOND Polymer Refine	no	no	DAB
114	Envision Flex TRS, High pH	30	SP1	1:50	THERMO FISHER	9101S1704E	30	DAKO OMNIS	N	N	Envision Flex DAB
120	Waterbath	20	alpha EP1	pre-dilute	Dako	10130087	20	EnVision Flex Dako AutostainerLink48	n	n	DAB
127	HIER	36 MIN	SP1	PREDILUTE	VENTANA	Y10757	32 MIN	ULTRAVIEW DAB	N	N	DAB
128	CC1	64 min	SP1	Pre-dilute	Ventana	Y25308	16 min	Ultraview	No	No	DAB
129	ER 2-high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1704S	15	Bond Refine Detection Kit	N	N	DAB
132	High pH	20	ER-EP1	RTU	Dako/Agilent	10127410	20	Envision Flex	N	N	DAB
133	HEIR	36 MIN	SP1	PREDILUTE	ROCHE	Y05956	32	POLYMER-ULTRAVIEW	N	N	DAB
134	HIER-CC1	30	SP1	RTU	VENTANA/ROCHE	G10362	8	ULTRAVIEW	N	N	DAB
138	HIER pH9	30	EP1	RTU	Dako	10132043	20	Polymer	N	N	DAB
141	HIER-CC1	30	SP1	RTU	VENTANA/ROCHE	G10362	8	ULTRAVIEW	N	N	DAB
144	CC1	24 min	SP1	1:50	ThermoScientific	1704s	16 min	Opti-view	no	Yes, Copper	DAB
147	HIER, PH9	20	SP1	1:150	Thermo-fisher	9101S1704E	15	POLYMER	N	N	DAB
148	CC1	36 min	SP1	predilute	Ventana	E03296	12 min	Ultraview	NO	NO	DAB
149	high pH OMNIS	30 min at 97 C	EP1	RTU	Dako Agilent	10132249	20	EnVision Flex	No	No	DAB
151	HIER2 pH 9	20	SP1	1:25	Thermo Scientific	9101S1704E	15	Bond Refine	N	N	DAB
175	HIER	36 mins	SP1	predilute	Roche	Y188880	32 mins	polymer	N	Y(copper)	DAB
178	HIER	32 MIN	SP1	NONE	VENTANA	N/A DISPENSER GONE	16	ULTRAVIEW DAB	N	N	DAB
183	Ultra CC1	36	SP1	RTU	Ventana Roche	Y07227	32	Ultraview	N	N	DAB
186	HIER	20 MIN.	SP1	1:50	THERMOSCIENTIFIC	9101S1704D	15 MIN.	POLYMER (LEICA)	N	N	DAB
187	CC1	16	SP1	Predilute	Roche	Y03296	8	Optiview	n	N	DAB
188	ER2	20	ER6F11	as per kit	Leica	51375	20	Refine Kit	N	N	DAB
189	CC1	64	SP1	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	SP1 rabbit	Pre-dilute	Ventana	Y18880	32	Ventana iView	N	N	DAB
192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	Y25308	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	SP1	RTU	ROCHE/VENTANA	Y24472	12	IVIEW	N	Y	DAB
196			SP1		VENTANA	Y18880	8	ULTRAVIEW DAB			
198	High pH HIER	30 min	EP1	Prediluted	Dako/Agilent	10133973	20 min	Envision Flex/ HRP	N	N	DAB
202	er1	20	6f11	1:75	leica	6053070	16	Refine Detection System	no	no	DAB
207	CC1-on line	36 minutes	SP1	Predilute	Ventana	Y24472	16 minutes	Ultraview	N	Y	DAB
209	HIEr high ph	20	EP1	none	Dako	10134722	30	Envision Polymer	none	none	DAB
221	pH6 Citrate Buffer	20	SP1	1:100	Cell Marque	1617304D	30	MACH4	N	N	DAB
230	HIER	64	SP1	predilute	Roche Diagnostics	Y25308	32	Ultraview	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	EnV FlexTRS, High PH	30 min	PR 1294	RTU	Dako	10129802	25 min	DAKO Envision Flex	N	N	DAB
102	DAKO PT - HIGH PH	20	16	1:200	NOVOCASTRA	6052038	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64	IE2	Pre	Ventana	Y12992	16	ULTRA VIEW	N	Y	DAB
106	CC1	64	PgR1294	220	Dako	10132621	32	Optiview	no	yes	DAB
107	Ultra cc1	64	PqR1294	1:50	Dako	10132621	32	Ultraview DAB	Y	N	DAB
109	HIER high pH- CC1	36 MIN	1294	1/50	DAKO	10132621	16 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	32	16	1/80	LEICA	6046807	30	OPTIVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	10 min	16	RTU	Leica	60534	15 min	BOND Polymer Refine	no	no	DAB
114	Envision Flex TRS, High pH	30	1294	RTU	DAKO	10126993	25	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	Waterbath	20	PgR 636	pre-dilute	Dako	10122270	20	EnVision Flex Dako AutostainerLin	n	y	DAB
127	HIER	36 MIN	IE2	PREDILUTE	VENTANA	Y00807	8 MIN	ULTRAVIEW DAB	N	N	DAB
128	CC1	64 min	IE2	Pre-dilute	Ventana	Y25295	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	High pH	20	PR-16	1:200	Leica	6044509	30	Envision Flex	N	N	DAB
133	HEIR	64	16	1:25	LEICA	6053394	60	POLYMER-ULTRAVIEW	N	N	DAB
134	HIER-CC1	30	IE2	RTU	VENTANA/ROCHE	Y12992	12	ULTRAVIEW	N	N	DAB
138	HIER pH9	30	1294	RTU	Dako	10129802	20	Polymer	N	N	DAB
141	HIER-CC1	30	IE2	RTU	VENTANA/ROCHE	Y12992	12	ULTRAVIEW	N	N	DAB
147	HIER, PH9	20	16	1:800	NCL	6027295	15	POLYMER	N	N	DAB
149	high pH OMNIS	30 min at 97 C	PgR636	RTU	Dako Agilent	10131210	10	EnVision Flex	No	No	DAB
151	HIER1 pH 6	20	16	1:100	Leica	6053394	15	Bond Refine	N	N	DAB
175	HIER	64 mins	IE2	predilute	Roche	Y21113	32 mins	polymer	N	Y(copper)	DAB
178	HIER	32 MIN	IE2	NONE	VENTANA	N/A DISPENS	16	ULTRAVIEW DAB	N	N	DAB
183	Ultra CC1	36	IE2	RTU	Ventana Roche	Y06097	32	Ultraview	n	n	DAB
186	HIER	20 MIN	PR88	1:50	BIOGENEX	MU328-UC	15 MIN.	POLYMER(LEICA)	N	N	DAB
187	CC1	64	IE2	Predilute	Roche	Y29059	12	Ultraview	N	N	DAB
188	ER1	20	312	as per kit	Leica	51462	15	Bond Polymer Refine Kit	N	N	DAB
189	CC1	64	IE2	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	Clone 16	1:50	Novocastra	6046807	32	Ventana iView	N	N	DAB
192	Ultra CC1	36 minutes	IE2	Ready to use	Ventana/Roche	Y21113	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	IE2	RTU	ROCHE/VENTANA	Y18852	20	IVIEW	N	Y	DAB
196			IE2		VENTANA	Y03229	8	ULTRAVIEW DAB	N	N	
198	High pH HIER	30 min	1294	1/100	Dako/Agilent	10121367	20 min	Envision Flex/ HRP	N	N	DAB
202	ER2	30	16	rtu	leica	60561	16	Refine Detection System	no	no	DAB
207	cc1-ON LINE	64 MINUTES	16	1/50	VECTOR	6031757	32 minutes	Ultraview	N	Y	DAB
209	HIER highph	20	PgR636	none	Dako	10134386	20	Envision Polymer	none	none	DAB
221	pH6 Citrate Buffer	20	1A6	1:400	Leica	6046807	30	MACH4	N	N	DAB
230	HIER	64	IE2	predilute	Roche Diagnostics	Y21113	16	Ultraview	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	EnV FlexTRS, High PH	30 min	HER 2 4B5	1:8	VENTANA	Y21121	15 min	DAKO Envision Flex	N	N	DAB
102	DAKO PT - HIGH PH	20	SP3	1:50	THERMO FISHER	9103S1701C1	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	36	4B5	PRE	Ventana	G09203	16	ULTRA VIEW	N	Y	DAB
106	CC1	36	4B5	predilute	Dako	Y28979	16	Ultraview	no	yes	DAB
107	Ultra cc1	64	4B5	Pre-diluted	Ventana	Y27661	24	Ultraview DAB	N	Y	DAB
109	HIER high pH-CC1	36 MIN	4B5	RTU	ROCHE	Y18581	16 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	36	4B5	PREDILUTE	VENTANA	Y27661	30	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 min	4B5	1:4 ratio of RTU	Ventana/Roche	G09203	15 min at RT	BOND Polymer Refine	no	no	DAB
114	Envision Flex TRS, High pH	30	4B5	1:8	Ventana Roche	Y12085	15	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	Waterbath	40	HER2 protein	pre-dilute	Dako	20049058	20	Herceptest-Dako Autostainer Link 48	n	n	DAB
127	HIER	36 MIN	4B5	PREDILUTE	VENTANA	Y21124	24 MIN	ULTRAVIEW DAB	N	N	DAB
129	ER 2- high pH retrieval	20	SP3	1:100	Thermo Scientific	SJ22462025	15	Bond Refine Detection Kit	N	N	DAB
133	HEIR	36	4B5	PREDILUTE	ROCHE	Y20145	24	POLYMER-ULTAVIEW	N	N	DAB
138	HIER pH6	20	HercepTest	RTU	Dako	20049058	30	Polymer / HercepTest kit	N	N	DAB
147	HIER PH9	20	SP3	1:100	Thermo	SA2327511	15	Polymer	N	N	DAB
149	high pH OMNIS	30 min at 97 C	AO485	1:600	Dako Agilent	20042564	20	EnVision Flex	No	No	DAB
151	HIER2 pH 9	20	SP3	1:75	Invitrogen	RK2291861	15	Bond Refine	N	N	DAB
175	HIER	32 mins	4B5	predilute	Roche	y21124	16 mins	polymer	N	Y(copper)	DAB
181	CC1	30 minutes	4B5	Pre-diluted	Ventana/Roche	Y20145	16 minutes	Ventana Ultraview DAB	no	yes	DAB
186	HIER	20 MIN	HER2	1:800	DAKO	20023582	15 MIN.	POLYMER(LEICA)	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	Y27661	24	Optiview	N	N	DAB
188	ER1	25	CB11	as per kit	Leica	52336		Oracle HER2 IHC System	N	N	DAB
189	CC1	32	4B5	pre-dilute	Ventana	unknown	12	ultraView DAB	N	N	ultraView DAB
190	CC1	32	SP3 rabbit	1:50	Thermofisher	9103S10701A	40	Ventana iView	Y	N	DAB
194	CC1	30	4B5	RTU	ROCHE/VENTANA	E01451	12	IVIEW	N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Roche/Ventana	Y27661	28 min	Ultraview	N	y	DAB
202	ER buffer from Herceptest	40	Her2	rtu	Agilent	20051074	30	Herceptest	no	no	DAB
207	CC 1-on line	36 minutes	4B5	Predilute	Ventana	Y28979	16 minutes	Ultraview	N	Y	DAB
221	Dako Visualization Solution	20	Dako Hercep Test	NEAT	Dako	20052480	30	Dako Kit	N	N	DAB
230	HIER	36	4B5	predilute	Roche Diagnostics	Y21124	16	Ultraview	N	N	DAB
237	Novocastra Epitope Retrieval Solutions	30	10A7	1:200	Leica	NCL-L-CBE-356	60	Novolink Polymer Detection Systems	N	N	DAB Substrate Buffer

Table S4. Descriptive statistics for ER based on cIQc assessment. Cores 21 and 26 were excluded from analysis due to technical issues.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	46	67.39	31	31/31 (100%)	1	1	1
102	46	78.26	36	35/36 (97%)	1	0.86	0.91
103	46	76.09	35	34/35 (97%)	1	0.83	0.89
106	46	80.43	37	36/37 (97%)	1	0.86	0.91
107	46	76.09	35	34/35 (97%)	1	0.86	0.91
109	46	73.91	34	33/34 (97%)	1	0.86	0.91
111	46	73.91	34	33/34 (97%)	1	0.86	0.91
112	46	63.04	29	28/29 (97%)	1	0.83	0.89
114	46	73.91	34	33/34 (97%)	1	0.86	0.91
120	46	76.09	35	34/35 (97%)	1	0.86	0.91
123	46	73.91	34	33/34 (97%)	1	0.86	0.91
125	46	73.91	34	33/34 (97%)	1	0.83	0.89
127	46	73.91	34	33/34 (97%)	1	0.86	0.91
128	46	69.57	32	32/32 (100%)	1	1	1
129	46	73.91	34	34/34 (100%)	1	1	1
132	46	71.74	33	32/33 (97%)	1	0.86	0.9
133	46	73.91	34	33/34 (97%)	0.96	1	0.91
134	46	69.57	32	31/32 (97%)	0.96	1	0.91
138	46	63.04	29	28/29 (97%)	1	0.86	0.9
141	46	67.39	31	30/31 (97%)	0.96	1	0.91
144	46	80.43	37	36/37 (97%)	0.97	1	0.92
147	46	73.91	34	34/34 (100%)	1	1	1
148	46	76.09	35	35/35 (100%)	1	1	1
149	46	69.57	32	32/32 (100%)	1	1	1
151	46	76.09	35	33/35 (94%)	0.96	0.86	0.82
175	46	73.91	34	34/34 (100%)	1	1	1
178	46	63.04	29	28/29 (97%)	0.96	1	0.9
183	46	76.09	35	34/35 (97%)	1	0.86	0.91
186	46	78.26	36	34/36 (94%)	0.97	0.86	0.82
187	46	73.91	34	33/34 (97%)	1	0.86	0.91
188	46	65.22	30	27/30 (90%)	1	0.57	0.67
189	46	69.57	32	32/32 (100%)	1	1	1
190	46	73.91	34	34/34 (100%)	1	1	1
192	46	71.74	33	31/33 (94%)	0.96	0.86	0.82
194	46	69.57	32	31/32 (97%)	1	0.86	0.9
196	46	71.74	33	33/33 (100%)	1	1	1
198	46	73.91	34	33/34 (97%)	0.96	1	0.91
199	46	71.74	33	32/33 (97%)	1	0.86	0.9
202	46	71.74	33	31/33 (94%)	1	0.71	0.8
207	46	76.09	35	35/35 (100%)	1	1	1
209	46	76.09	35	34/35 (97%)	0.96	1	0.92
221	46	78.26	36	35/36 (97%)	0.96	1	0.92
230	46	73.91	34	33/34 (97%)	1	0.86	0.91
233	46	80.43	37	35/37 (95%)	0.93	1	0.84

Table S5. Descriptive statistics for PR based on cIQc assessment. Cores 12 and 26 were excluded from analysis due technical issues.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	44	72.73	32	30/32 (94%)	0.89	1	0.88
102	44	81.82	36	36/36 (100%)	1	1	1
103	44	81.82	36	36/36 (100%)	1	1	1
106	44	79.55	35	35/35 (100%)	1	1	1
107	44	75	33	33/33 (100%)	1	1	1
109	44	77.27	34	34/34 (100%)	1	1	1
111	44	77.27	34	34/34 (100%)	1	1	1
112	44	61.36	27	27/27 (100%)	1	1	1
114	44	75	33	32/33 (97%)	0.94	1	0.94
120	44	79.55	35	35/35 (100%)	1	1	1
123	44	75	33	32/33 (97%)	0.94	1	0.94
125	44	70.45	31	30/31 (97%)	0.94	1	0.94
127	44	75	33	31/33 (94%)	1	0.86	0.87
128	44	72.73	32	30/32 (94%)	1	0.86	0.87
129	44	75	33	33/33 (100%)	1	1	1
132	44	72.73	32	32/32 (100%)	1	1	1
133	44	75	33	33/33 (100%)	1	1	1
134	44	65.91	29	29/29 (100%)	1	1	1
138	44	61.36	27	27/27 (100%)	1	1	1
141	44	75	33	30/33 (91%)	1	0.79	0.81
147	44	72.73	32	32/32 (100%)	1	1	1
149	44	61.36	27	26/27 (96%)	0.94	1	0.92
151	44	77.27	34	34/34 (100%)	1	1	1
175	44	75	33	31/33 (94%)	1	0.87	0.88
178	44	68.18	30	30/30 (100%)	1	1	1
183	44	75	33	29/33 (88%)	1	0.71	0.74
186	44	84.09	37	33/37 (89%)	1	0.79	0.78
187	44	77.27	34	27/34 (79%)	1	0.56	0.58
188	44	68.18	30	30/30 (100%)	1	1	1
189	44	68.18	30	29/30 (97%)	1	0.92	0.93
190	44	75	33	31/33 (94%)	0.89	1	0.88
192	44	72.73	32	32/32 (100%)	1	1	1
194	44	72.73	32	30/32 (94%)	0.89	1	0.88
196	44	77.27	34	32/34 (94%)	1	0.87	0.88
198	44	75	33	31/33 (94%)	0.88	1	0.88
199	44	79.55	35	35/35 (100%)	1	1	1
202	44	72.73	32	32/32 (100%)	1	1	1
207	44	65.91	29	26/29 (90%)	0.82	1	0.79
209	44	77.27	34	34/34 (100%)	1	1	1
230	44	72.73	32	32/32 (100%)	1	1	1

Table S6. Descriptive statistics for HER2 based on cIQc assessment. Core 15 and 38 were excluded from analysis.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	44	59.09	25	25/25 (100%)	1	1	1
102	44	59.09	25	25/25 (100%)	1	1	1
103	44	68.18	26	26/26 (100%)	1	1	1
106	44	70.45	28	28/28 (100%)	1	1	1
107	44	68.18	25	25/25 (100%)	1	1	1
109	44	59.09	25	25/25 (100%)	1	1	1
111	44	68.18	27	27/27 (100%)	1	1	1
112	44	56.82	24	24/24 (100%)	1	1	1
114	44	75	28	28/28 (100%)	1	1	1
120	44	65.91	26	26/26 (100%)	1	1	1
123	44	65.91	27	27/27 (100%)	1	1	1
125	44	68.18	26	26/26 (100%)	1	1	1
127	44	63.64	26	26/26 (100%)	1	1	1
129	44	65.91	26	26/26 (100%)	1	1	1
133	44	68.18	26	26/26 (100%)	1	1	1
138	44	59.09	24	24/24 (100%)	1	1	1
147	44	65.91	26	26/26 (100%)	1	1	1
149	44	63.64	27	27/27 (100%)	1	1	1
151	44	61.36	26	26/26 (100%)	1	1	1
175	44	63.64	25	25/25 (100%)	1	1	1
181	44	56.82	23	23/23 (100%)	1	1	1
186	44	70.45	28	28/28 (100%)	1	1	1
187	44	63.64	25	25/25 (100%)	1	1	1
188	44	56.82	22	22/22 (100%)	1	1	1
189	44	79.55	28	28/28 (100%)	1	1	1
190	44	68.18	28	28/28 (100%)	1	1	1
194	44	70.45	28	28/28 (100%)	1	1	1
198	44	65.91	27	27/27 (100%)	1	1	1
199	44	68.18	27	27/27 (100%)	1	1	1
202	44	63.64	27	27/27 (100%)	1	1	1
207	44	65.91	27	27/27 (100%)	1	1	1
221	44	63.64	26	26/26 (100%)	1	1	1
230	44	61.36	25	25/25 (100%)	1	1	1
233	44	81.82	27	27/27 (100%)	1	1	1
237	44	90.91	27	27/27 (100%)	1	1	1