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Assessors' report for cIQc Run 50: Breast Module (ER, PR and HER2)

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Assessment performed on October 8, 2015 and October 14, 2015 at Vancouver General Hospital.

Overview

Similar to cIQc Run 27 from 2012, Run 50 consisted of a duplicate core tissue microarray containing 40 breast cancer cases (20 triple negative and 20 non-triple negative according to pathology charts) with accompanying PAM50 molecular subtype assignment. With the use of a tissue microarray enriched for basal-like breast carcinoma cases, higher variation in the breast cancer biomarkers was observed in cIQc Run 27. It is believed that such variation in ER can be particularly attributed to more sensitive ER testing in current use; this is of potential clinical significance as basal-like carcinomas are responsive to chemotherapy, but not tamoxifen, and ER positivity in these cases could result in inappropriate treatments being used.

ER

Consistent with cIQc Run 47, the trend of weak ER positive cases (i.e. Allred scores 3-5) was especially evident in this particular quality assurance challenge due to the tissue microarray being enriched for triple negative cases. Most notably, cores 61/62 and 67/68 possessed this weak positive staining that would be considered an Allred 3 or 4 in clinical practice. Cores 41/42, 49/50 and 75/76 were excluded from statistical analyses due to high dropout or the presence of only a few tumour cells, leading to considerable heterogeneity. Overall, it was noted that labs using the EP1 clone had weaker staining. Sites using the iView detection system also had generally weaker staining when using the SP1 RTU, while labs following the package insert using ultraView had optimal staining. See protocol for Lab 189 in Table S1.

Slides from Labs 152, 155, 160, 177, 184/184a and 202 were not returned in time for the assessment meeting. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Optimal	
102	Sub-optimal	False +ve staining in cores 11/12; weak staining observed in cores that are consistently -ve in other labs
106	Optimal	Two slides submitted for review using different clones; staining similar but SP1 noted to be slightly more intense than 6F11
107	Optimal	
109	Optimal	
111	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	
115	Optimal	



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116	Optimal	
119	Optimal	
120	Optimal	
122	Optimal	
123	Optimal	
124	Optimal	
125	Optimal	
126	Optimal	
127	Optimal	
128	Optimal	
129	Optimal	
132	Optimal	
133	Optimal	
134	Adequate	Generally weak staining compared to other labs
135	Fail	Intense nuclear staining; 4 strongly false +ve cores. May require greater dilution of primary antibody (see similar protocol used by Lab 112).
138	Adequate	Generally weak staining compared to other labs
139	Adequate	Generally weak staining compared to other labs
141	Optimal	
143	Optimal	
144	Optimal	
145	Optimal	
146	Adequate	Slight background staining; dark counterstain interfered with interpretation of weak positive cases
147	Optimal	
148	Optimal	
149	Adequate	Generally weak staining compared to other labs
150	Optimal	
151	Optimal	
152	--	No slides
153	Optimal	
155	--	No slides
157	Optimal	



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159	Adequate	Generally weak staining compared to other labs; dark counterstain interfered with interpretation of weak positive cases
160	--	No slides
161	Adequate	Generally weak staining compared to other labs; dark counterstain interfered with interpretation of weak positive cases
162	Optimal	
164	Adequate	Generally weak staining compared to other labs
165	Optimal	
167	Fail	Very weak staining overall leading to false -ve cores; assessors suspect a possible error in the antibody dispenser
168	Sub-optimal	3 false +ve cores observed
170	Adequate	Generally weak staining compared to other labs
173	Optimal	
175	Adequate	Generally weak staining compared to other labs
177	--	Slides not available
178	Optimal	
180	Optimal	
183	Optimal	
184	--	No slides
184a	--	No slides
186	Optimal	
187	Optimal	
188	Optimal	
189	Optimal	
190	Optimal	
191	Optimal	
192	Optimal	
194	Optimal	Slide overdigested leading to multiple unsatisfactory results
196	Optimal	
198	Optimal	
199	Optimal	
202	--	No slide
207	Optimal	Noted to have exceptionally nice staining
208	Sub-optimal	Extremely faint blush in false +ve (equivocal) cores 55/56/58; no staining in weak positive cores 61/62 or 67/68



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209	Optimal	
215	Optimal	
216	Optimal	
217	Adequate	False +ve staining in cores 71/72 that are consistently -ve in other labs
221	Optimal	

*Based on cIQc assessor consensus

PR

Cores 41, 42, 44 and 75 were excluded from statistical analyses due to high core dropout or lack of tumour. Tumour heterogeneity was particularly evident in cores 47/48 and 51/52. Slides from Labs 152, 155, 160, 177, 184/184a and 202 were not returned in time for the assessment meeting. Overall, it was noted that labs using an Envision Flex detection system had cytoplasmic background staining. Since the majority of labs exhibited optimal/adequate staining, participant specific feedback is provided below only for labs for which a comment was made by the assessment team:

Lab	IHC Status*	cIQc Comments
120	Adequate	Cytoplasmic background staining that possibly interfered with self-assessment; multiple positive cores by self-assessment were changed to negative after cIQc assessment.
139	Adequate	Generally weak staining compared to other labs.
146	Adequate	Cytoplasmic background staining observed.
159	Adequate	Cytoplasmic background staining observed.
161	Adequate	Cytoplasmic background staining observed.
165	Failed	Significant background staining; many positive cores that are consistently negative in other labs.
168	Adequate	Cytoplasmic background staining observed.
192	Sub-optimal	Weak blush staining leading to positive cores that are consistently negative in other labs.
209	Adequate	Cytoplasmic background staining observed.
217	Failed	Positivity observed in many cores that are consistently negative in other labs.

*Based on cIQc assessor consensus



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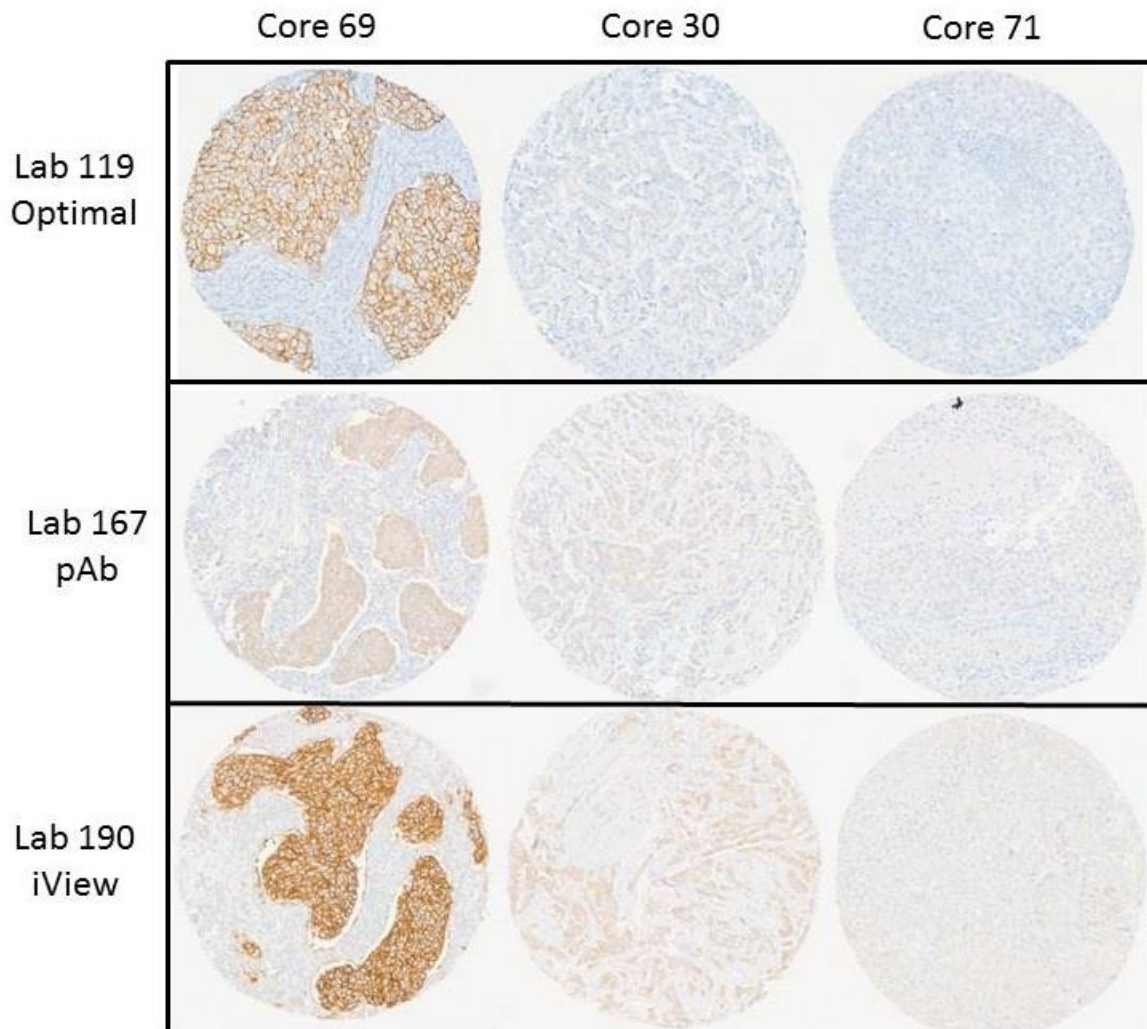
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HER2

Cores 7, 41, 42, 44 and 75 were excluded from statistical analyses due to high core dropout. Slides from Labs 152, 155, 160 and 202 were not returned in time for the assessment meeting. Overall, two issues were observed:

- 1) The iView detection system yields less optimal staining overall compared to other protocols despite correct interpretation by labs using this detection system. Specifically, staining intensity can be weaker and increased non-specific staining was observed.
- 2) Monoclonal antibody clones provide a superior dynamic range and yield less indeterminate results than polyclonal antibodies. While the cost is greater, labs are likely to compensate and possibly see savings in the reduced number of cases sent for HER2 FISH testing.

Representative staining of such issues is illustrated below:





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Participant-specific feedback for HER2 IHC is summarized below:

Lab	IHC Status*	CIQC Comments
101	Optimal	
102	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
111	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	
115	Adequate	Generally weak staining compared to other labs.
116	Optimal	
119	Optimal	Exceptionally nice staining
120	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
123	Optimal	
124	Optimal	
125	Optimal	
126	Optimal	
127	Optimal	
129	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
133	Optimal	
135	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
138	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
139	Optimal	
145	Optimal	Slight background staining
147	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs. NOTE: Slide submitted using new SP3 protocol shows optimal staining.
149	Optimal	
150	Optimal	
151	Sub-optimal	Significantly greater staining of non-amplified cases compared to other labs. 5 cores were changed from 3+ to 2+ after CIQC assessment.
152	--	No slide



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153	Optimal	
155	--	No slide
157	Optimal	
160	--	No slide
161	Failed	Both weak staining and weak counterstain, leading to a lack of sensitivity and specificity.
162	Optimal	
164	Optimal	Slightly weaker staining compared to other labs.
167	Failed	Generally very weak staining; amplified cases are weakly stained yet significant staining of non-amplified cases still observed.
168	Optimal	
170	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
175	Optimal	Slightly weaker staining compared to other labs.
181	Optimal	
186	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
187	Optimal	
188	Optimal	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
189	Optimal	
190	Sub-optimal	Significant non-specific staining observed
191	Optimal	
194	Optimal	
198	Optimal	
199	Optimal	7 cores were changed from 3+ to 2+ after CIQC assessment; over-interpretation of 3+ by self-assessment.
202	--	No slide
207	Optimal	
215	Optimal	
216	Optimal	
217	Adequate	Interpretation correct but significantly greater staining of non-amplified cases compared to other labs.
221	Sub-optimal	3 false 3+ cores; significantly greater staining of non-amplified cases compared to other labs.

*Based on CIQC assessor consensus



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Garratograms after cIQc assessment of ER, PR and HER2 are provided in Supplementary Figures 1 to 3. Supplementary Tables 1 to 3 summarizing staining protocols and Supplementary Tables 4 to 6 summarizing descriptive statistics can also 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 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.

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 minutes	SP1	1:50	Thermo	9101S1406E	32 minutes	OptiView	N	Y	DAB
102	Dako 3in1 High pH	10/20/10	SP1	1:35	Labvision (Thermo)	9101S1406K	30 min RT	DAKO FLEX	NO	YES	DAB+
106	CC1	64 min	SP1	predilute	Ventana Roche	Bo3602	32 min	Optiview	no	yes	DAB
107	Ultra cc1	36	SP1	pre-diluted	Ventana	F00389	16	ultraview DAB	N	Y	DAB
109	HIER - HIGH pH	64 MIN	SP-1	RTU	VENTANA-ROCHE	E06774	32 MIN	ULTRAVIEW	NO	YES	DAB
111	CC1	36 MIN	SP1	pre-diluted	VENTANA	2226	32 MIN	ULTRAVIEW	N	Y	DAB/COPPER
112	BOND ER2 pH 9.0	20 minutes	SP1	1:150	ThermoFisher	PI1914231	15 minutes @ RT	Bond Polymer Refine	NO	NO	DAB
113	CC1	32'	SP1	1/70	ThermoScientific	9101S1501A	32'	Optiview	N	Y	DAB
114	CC1	32	SP1	1:50	THERMO FISHER	9101S1406K	16	OPTVIEW	N	Y	DAB
115	CC1	30min	SP1	Prediluted	Ventana	F02226	32	IVIEW DAB	Y	Cooper	DAB
116	CC1	64 MIN	SP1	RTU	VENTANA	E09654	32 MIN	ultraView DAB Ventana	N	N	DAB
119	HIER/CC1	30 minutes	SP1	pre-diluted	Ventana	F02226	24 minutes	Ultraview	--	--	DAB
120	waterbath	20	EP1	ready to use	Dako	10095094	20	EnVision Flex+	n	n	DAB
122	HIER	ER2-20min	SP1	Ready to use	Ventana	E06774	15min	Polymer refine	N	N	DAB
123	CC1	30 MIN	SP1	PREDILUTE	ROCHE/VENTANA	F00389	32 MIN	ULTRAVIEW	NO	YES	DAB
124	CC1	60	SP1	1/100	Cell Marque	1422401B	32	Dab IView	n	n	DAB
125	Bond ER2	20	SP1	predilute	Ventana	F02583	15	Bond Polymer Refine	N	Y	DAB
126	steamer, citrate pH6.0	20' at 99deg C	SP1	1:200	Thermo Scientific	9101S1501A	30'	Dako Envision small polymer	N	N	DAB
127	automated (ultra cell conditioner 1)	36 Min.	SP1	Predilute	Ventana	E02456	32 Min.	Ultra View DAB	N	N	DAB
129	ER2 - high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1501A	15	Bond Polymer Refine	N	N	DAB
132	Flex High pH	20	EP1	RTU	Dako	10095666	20	Flex	N	N	DAB
133	CC1 HIER	36 minutes	SP1	Predilute	Ventana	F07165	32 minutes	Ultraview	n	n	Dab
134	HIER	38 minutes	SP1	Predilute	Roche	E09654	8 minutes	Ultraview Universal	N	N	DAB
135	EDTA buffer PH9	20 min	SP1	1:50	Thermal Fisher	9101S1210L	15 min	Bond Polymer Refine	N	N	DAB
138	HIER pH9	20	EP1	RTU	Dako	10098852	20	Polymer	N	N	DAB
139	HIER automated	30	sp1	ready use	Ventana	F02226	32	Iview	N	N	DAB
141	HIER	30 min	SP1	RTU	Ventana/Roche	E09654	8 min	ultraView	N	N	DAB
143	HIER, automated online system	> 30 but < 45 min	SP1	no	confirm Ventana / Roche	E06774	16 min	Optiview detection kit	NO	Yes	DAB
144	CC1	24 min	SP1	pre-dilute	ThermoScientific	91015140	16 min	Optiview	No	Yes - copper	DAB
145	CC1	32	SP1	1/100	CELLMARQUE	1316403E	28	XT OPTIVIEW ihc v4	No	No	DAB
146	Flex TRS high	20	EP1	RTU	Dako	10090685	20	EnVision Flex	n	n	DAB
147	HIER, pH9	20	SP1	1:150	Thermo-Fisher	9101S1501A	15	Polymer	N	N	DAB
148	CC1	36 min	SP1	RTU	Ventana	F02226	8 min	Ultraview DAB	N	Y	DAB
149	PT Link high pH 97 C	20	EP1	RTU	Dako	10097676	20	EnVision Flex	No	No	DAB
150	cc1	30min	sp1	n/a	ventana	0	16 min	ultraview	n	y	dab
151	BUFFER pH9.0	20 min	SP1	1:100	THERMO	9101S1501A	15 MIN	Bond Polymer Refine	N	N	DAB
152	HIER	60 minutes	SP1	Pre-diluted	Roche Ventana	E07165	16 minutes	IView	No	Yes	DAB
153	CC1	32	SP1	s/o	Ventana	e07165	8	optiview dab	N	Y	DAB
155	CC1	30	SP1	Pre-diluted	Ventana	E10753	40	Ultraview dab	n	n	Dab
157	CC1	24 MIN.	SP 1	Pre-diluted	VENTANA	E 02453	24 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
159	High pH, automated	40 min total	EP1	Pre-diluted	DAKO	10098852	20	Envision	No	No	DAB
160	EDTA pH8	36	SP1	No	Ventana	?	8	Ventana Ultraview	N	N	DAB
161	HIER-High EDTA TRIS	20 Minutes	EP1	RTU	DAKO	10100789	20 Minutes	Envision Flex	no	no	DAB
162	CC1 Benchmark	48 min	SP1	1:100	Thermo Scientific	9101s1406L	32 min	OptiView	-	-	Dab
164	ultra CC1	36	SP1	predilute	Ventana	F02226	8	Ultraview	N	N	DAB
165	cc1	30	sp1	nil	ventana	e10753	28 min	ultra view	n	n	dab
167	CC1	30 min	SP1	pre diluted	Roche	D08925	8 min	Ultraview DAB kit	N	Y (copper)	DAB
168	HIER	48 min	SP1	1/100	Cellmarque	1422401B	30	Envision Flex +	N	Y	DAB
170	EDTA ph 9.0 HIER	20 min	Ep1	ready to use	Dako	10098852	20 min	Envision Flex Dako	no	no	DAB
173	CC1-EDTA	30 min.	SP1	pre-diluted	Ventana	E02453	16 Min.	Ultraview-polymer	N	Y	DAB
175	HIER	36	SP1	Pre-dilute	Roche	F02226	32	Ultraview	N	Y	DAB
177	CC1	30 min	6F11	1:25	Novocastra	--	32 min	Ultraview	y	y	DAB
178	HIER	32	SP1	None	Ventana	E08617	16	Ultraview	N	N	DAB
180	Ventana CC1	32 min	SP1	RTU	Ventana	E02453	16 min	Optiview	N	N	DAB
183	Ultra CC1	36	SP1	predilute	Roche	E06774	32	Ultraview	n	N	DAB
186	HEIR	20 minutes	SP1	1:50	Thermo Scientific	9101S1406H	15 minutes	Bond Polymer	N	N	DAB
187	CC1	16	SP1	Predilute	Roche	E10753	8	Optiview	N	N	DAB
188	Bond Epitope Retrieval Solution 1	20	6F11	1/50	Leica	6027092	15	Bond Polymer Refine	Y	N	DAB
189	CC1	8-64-8	SP1	Pre-dilute	Roche/Ventana	unknown	16	Ultraview	N	N	DAB
190	CC1 mild	?	SP1	prediluted	ventana	DO7686	32	IVIEW	N	none	DAB
191	CC1	60'	SP1	RTU	Roche	E02456	16'	ultraview DAB	N	N	DAB
194	CC1	30	SP1	PREDILUTE	VENTANA	E04942	12	IVIEW KIT(AVIDIN-BIOTIN)	Y	Y	DAB
196	DAB	8	SP1		VENTANNA	F02226					
198	Citrate pH 6.2 pressure cooker in microwave	5 min	6F11	1/100	Leica	6027943	30 min	Mach 1 polymer	Y	Y	DAB
199	HIER	20	6F11	RTU	Leica	30295	15	Lecia Bond Refine	N	N	DAB
202	Bond ER1 solution pH6	20 min	6F11	1/50	Leica	6027943	15 min	Leica Refine Detection kit	n	n	dab
207	CC1-	36 MINUTES	SP1	Prediluted	Ventana	F00389	16 minutes	UltraView DAB	N	N	DAB
208	HEIR citrate	30	6F11	1/50	Leica	6027943	60	Envision Flex	N	N	DAB FLEX
209	HIER	20 @ 97C and 20 cool to 85C	EP1	Pre dilute	Dako	10100789	20mins	Envision+	N	N	DAB
215	CC1	64 mins	SP1	Predilute	Roche	F02226	32 mins	Ultraview	N	N	DAB
216	HIER	30	SP1	Predilute	VENTANA/ROCHE	F02226	24	AVIDIN/BIOTIN (IVIEW DAB)	N	Y	DAB
217	CC1	64	SP1	Pre-dilute	Roche-Ventana	E10753	20	Optiview	No	No	DAB
221	pH6 Citrate Buffer	20	SP1	1:100	Cell Marque	1422401B	30	Rabbit EnVision	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 minutes	16	1:100	Leica	6015355	32 minutes	OptiView	N	Y	DAB
102	DAKO 3in1 High pH	10/20/10	16	1:150	Labvision (Thermo)	6027462	30 min RT	Dako FLEX	No	Yes	DAB+
106	Microwave pressure cooker	3	PgR 1294	1:1500	Dako	10085593	60	Elite	no	no	DAB
107	Ultra cc1	64	PgR 1294	1:50	Dako	10100055	32	ultraview DAB	Y	Y	DAB
109	HIER HIGH pH	36 MIN	PgR1294	1/50	DAKO	16082389	16 MIN	ULTRAVIEW	NO	YES	DAB
111	CC1	48 MIN	16	1/80	LEICA	6027462	32 MIN	OPTIVIEW	N	Cu	DAB
112	BOND ER2 pH 9.0	12 minutes	16	RTU	LEICA	25509	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
113	Low pH	30'	Pgr 636	1/300	Dako	10086359	10'	Flex +20	Y	N	DAB
114	CC1	32	16	1:25	NOVOCASTRA	6027462	16	OPTIVIEW	N	Y	DAB
115	CC1	30min	1E2	Prediluted	Ventana	F00256	32min	VIEW DAB	Y	Cu	DAB
116	CC1	64 min	clone 16	1/100	Leica	6027462	48 min	ultraView DAB Ventana	Y	.	DAB
119	HIER/CC1	30 minutes	1E2	pre-diluted	Ventana	F00256	16 minutes	Ultraview	--	--	DAB
120	waterbath	20	PgR636	RTU	Dako	10096659	20	EnVision Flex+	y	n	DAB
122	Hier	ER2-20	16	RTU	Leica NovaCastra	29086	15min	Polymer refine	N	N	DAB
123	CC1	60 MIN	16	1/50	VECTOR	6031757	32 MIN	ULTRAVIEW	NO	YES	DAB
124	CC1	30	1E2	Predilute	E00256	Ventana	20	Dab IView	n	n	Dab
125	Bond ER2	20	16	predilute	Leica	27787	15	Bond Polymer Refine	N	Y	DAB
126	steamer, citrate pH6.0	20' at 99deg C	PgR636	1:500 0.16ug/ml	Dako	10090531	30'	Dako Envision small polymer	N	N	DAB
127	automated (ultra cell conditioner 1)	36 Min.	1E2	Predilute	Ventana	E04627	8 Min.	Ultra View DAB	N	N	DAB
129	ER2 - high pH retrieval	20	16	1:400	Novocastra	Z050510	15	Leica Bond Refine Kit	n	n	DAB
132	Flex High pH	20	PR16	1:200	Vector	6024555	30	Flex	N	N	DAB
133	CC1 HIER	64 minutes	16	1/25	Vector	6031757	60 minutes	Ultraview	n	n	dab
134	HIER	38 minutes	1E2	Predilute	Roche	EO2706	12 minutes	Ultraview Universal	N	N	DAB
135	EDTA buffer pH9	20 min	16	1:400	Leica	1:400	15 minm	Bond polymer refine detection	N	N	DAB
138	HIER pH9	20	636	RTU	Dako	10098331	20	polymer	N	N	DAB
139	HIER automated	30	1E2	RTU	Ventana	F00256	32	Iview	N	N	DAB
141	HIER	30 mn	1E2	RTU	Ventana/Roche	E02706	12 min	ultraView	N	N	DAB
143	HIER, automated online system	> 30 but < 45 min	1E2	no	confirm Ventana / Roche	E09558	24 min	Optiview detection kit	NO	Yes	DAB
145	CC1	32	16	1/100	NOVOCASTRA	6027462	28	XT OPTIVIEH ihc v4	No	NO	DAb
146	Flex TRS High	20	636	RTU	Dako	10099986	30	EnVision Flex	n	n	DAB
147	HIER, pH9	20	16	1:800	Novovastra	6027295	15	Bond Refine Polymer	N	N	DAB
149	PT Link high pH 97 C	20	PGR 636	RTU	Dako	10099684	20	EnVision Flex	Yes	No	DAB
150	cc1	30min	1E2	n/a	ventana	0	20min	ultraview	n	y	dab
151	BUFFER pH 6.0	20 MIN	1A6	1:200	LEICA/NCL	6027295	15 MIN	BOND REFINE POLYMER	N	N	DAB
152	HIER	60 minutes	1E2	Pre-diluted	Roche Ventana	E06575	32 minutes	IView	No	Yes	DAB
153	CC1	32	1E2	S/O	VENTANA	E09555	24	OPTIVIEW DAB	N	Y	DAB
155	CC1	30	1E2	Predilute	Ventana	E09558	32	Ultraview dab	n	n	dab
157	CC 1	24 MIN.	IE 2	Predilute	VENTANA	E 02707	24 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
158											
159	High pH, automated	40 min total	PgR636	prediluted	DAKO	10095511	30 min	Envision	no	no	DAB
160	EDTA pH8	36	1E2	No	Ventana	?	8	Ventana Ultraview	N	N	DAB
161	HIER-High EDTA TRIS tampon	20 minutes	PgR636	RTU	DAKO	10101557	20 Minutes	Envision Flex	no	mouse linker	DAB
162	CC1, Ventana Benchmark	48 min	16	1:80	Leica	6020162	32 min	OptiView Dab, Ventana	-	-	OptiView Dab, Ventana
164	CC1	36	1E2	predilute	Ventana	F00256	8	Ultraview	N	N	DAB
165	cc1	30	1E2	nil	ventana	f00256	24	ultra view	n	n	dab
167	CC1	30 min	1E2	pre diluted	Roche	D09110	8 min	Ultraview DAB kit	N	Y (copper)	DAB
168	HIER	48 min	PgR636	RTU	Dako	10101557	20	Envision Flex +	N	Y	DAB
170	EDTA ph 9.0 HIER	20 min	PJR636	RTU	Dako	10101557	20 min	Envision Flex Dako	no	no	DAB
173	CC1-EDTA	30 min.	1E2	pre-diluted	Ventana	D09634	16 min.	Ultraview-polymer	N	Y	DAB
175	HIER	64	1E2	Pre-dilute	Roche	F00256	32	Ultraview	N	Y	DAB
177	CC1	32 min	PgR636	1:25	Dako	--	32 min	Ultraview	y	y	DAB
178	HIER	32	1E2	None	Ventana	F00256	16	Ultraview	N	N	DAB
183	Ultra CC1	36	1E2	predilute	Roche	E04944	32	ultraview	N	N	DAB
186	HEIR	20 minutes	PR88	1:100	BioGenex	MU3281213	15 minutes	Bond Polymer	N	N	DAB
187	CC1	64.0	1E2	Predilute	Roche	F00256	12	Optiview	N	N	DAB
188	Bond Epitope Retrieval Solution 2	20	16	RTU	Leica	30158	15	Bond Polymer Refine	Y	N	DAB
189	CC1	8-64-8	1E2	Pre-dilute	Roche/Ventana	unknown	16	Ultraview	N	N	DAB
190	CCI mild	?	16	1:50	Novocastra	6015355	32	Iview	N	N	DAB
191	CC1	60'	1E2	RTU	Roche	E04627	16'	ultraview DAB	N	N	DAB
192	Heat Induced	16	1E2	0.05 M	Roche Canada	E09558					
194	CC1	30	1E2	Predilute	VENTANA	E06575	20	IVIEW KIT (AVIDIN-BIOTIN)	N	Y	DAB
196	DAB	8	1E2		VENTANNA	E09558					
198	citrate pH 6.2 pressure cooker in microwave	5 min	16	1/600	Vector Laboratories	6024555	30 min	MACH 1 polymer	Y	Y	DAB
199	HIER	20	16	1:200	Leica	6027462	15	Leica Bond Refine	N	N	DAB
202	Bond ER2 solution pH 9.5	30 min	160E	RTU	Leica	40633	15 min	Leica Refine Detection kit	n	n	dab
207	CC1	36 minutes	1E2	Prediluted	Ventana	E09555	16 minutes	Ultraview DAB	N	N	DAB
208	HEIR EDTA	30	PgR 636	Predilute	DAKO	10101557	60	ENVISION FLEX	N	N	DAB FLEX
209	HIER	20 @ 97C and 20 down to 85C	PgR 636	Pre dilute	Dako	10101557	20mins	Flex+	N	N	DAB
215	CC1	64 mins	1E2	Predilute	Roche	F00256	16 mins	DAB	No	NO	DAB
216	HIER	30	1E2	Predilute	VENTANA/ROCHE	F00256	24	AVIDIN/BIOTIN (IVIEW DAB)	N	Y	DAB
217	CC1	64	1E2	pre-dilute	Roche-Ventana	E09558	16	Optiview	No	No	DAB
221	pH6 Citrate Buffer	20	1A6	1:200	Leica	6027462	30	Mouse EnVision	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	CC1	32 minutes	SP3	1:200	Thermo	9103S1305I	32 minutes	OptiView	N	Y	DAB
102	Dako 3in1 High pH	10/20/10	SP3	1:750	Labvision (Thermo)	9103S1306P	30 min RT	Dako FLEX	No	Yes	DAB+
106	CC1	36 min	4B5	predilute	Ventana Roche	C01215	16 min	ultraview	no	yes	DAB
107	Ultraview cc1	36	4B5	pre-diluted	Ventana	F03192	8	ultraview DAB	N	Y	DAB
109	HIER HIGH pH	36 MIN	4B5	RTU	VENATANA-ROCHE	F00396	16 MIN	ULTRAVIEW	NO	YES	DAB
111	CC1	36 MIN	4B5	predilute	VENTANA	F00265	32 MIN	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	4B5	1:3 ratio of the RTU	Ventana	E00842	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
113	CC1	30'	SP3	1/50	CellMarque/ESBE	1332302B	16'	Ultraview	N	Y	DAB
114	CC1	32	SP3	1:200	THERMO FISHER	9103S1202E	16	OPTIVIEW	N	Y	DAB
115	CC1	30min	4B5	Prediluted	Ventana	F00396	24 min	IVIEW DAB	Y	Cu	DAB
116	CC1	40 min	SP3	1/100	Thermo Scientific	1306 F	36 min	Optiview DAB Ventana	N	.	DAB
119	HIER/CC1	30 minutes	4B5	pre-diluted	Ventana	E10786	16 minutes	Ultraview	--	--	DAB
120	waterbath	40	?	ready to use	Dako	20018799	?	HercepTest	n	n	DAB
123	CC1	30 MIN	4B5	predilute	ROCHE/VENTANA	E10786	32 MIN	ULTRAVIEW	NO	YES	DAB
124	CC1	30	4B5	predilute	Ventana	E10786	12	Ultra View	n	n	DAB
125	Ventana CC1	36	4B5	predilute	Ventana	F00396	16	Ventana UltraView	N	N	DAB
126	Steam, Tris pH10.0	30' at 99deg C	SP3	1:400	NeoMarkers	9103S1407L	30'	Dako Envision small polymer	N	N	DAB
127	automated (ultra cell conditioner 1)	36 Min.	4B5	Predilute	Ventana	F00396	24 Min.	Ultra View DAB	N	N	DAB
129	ER1 - low pH retrieval	20	Cerb b2	1:600	Dako	86782	15	Leica Bond Refine Kit	n	n	DAB
133	CC1 HIER	36 minutes	4B5	predilute	Ventana	F00365	24 minutes	Ultraview	n	n	dab
135	citrate buffer PH 6	20 min	polyclonal	1:700	Dako A0485	81393	15 min	Bond polymer refine detection	N	N	DAB
138	HIER pH6	40	HercepTest	RTU	Dako	20018877	30	Polymer	N	N	DAB
139	HIER automated	32	4B5	ready use	Ventana	F03192	48	Iview	N	N	DAB
145	CC1	32	SP3	1/300	CELLMARQUE	1332302A	16	XT OPTIVIEW ihc v4	No	No	DAB
147	HIER, pH6	20	Polyclonal	1:400	Dako	20002512A	15	Bond Refine Polymer	N	N	DAB
149	PT Link high pH 97 C	20	SP3	1:100	Thermo	00S1407A	30	EnVision Flex	Yes	No	DAB
150	cc1	30min	SP3	1/100	neomarkers	0	20min	ultraview	n	y	dab
151	BUFFER pH 6.0	20 MIN	POLYCLONAL	1:500	DAKO	2002512 A	15 MIN	BOND REFINE POLYMER	N	N	DAB
152	HIER	60 minutes	4B5	Pre-diluted	Roche Ventana	F00396	32 minutes	IView	No	Yes	DAB
153	CC1	32	4B5	S/O	vENTANA	E10257	12	OPTIVIEW DAB	N	Y	DAB
155	CC1	30	4B5	predilute	Ventana	E10257	32	Ultraview dab	n	n	dab
157	CC1	24 MIN.	4B 5	predilute	VENTANA	E 04951	24 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
160	EDTA pH8	36	A0485	No	Dako	?	32	Ventana ultraview	N	N	DAB
161	Herceptest epitope	40 minutes	Rabbit anti-HER2	RTU	DAKO	20020503	30 minutes	Herceptest visualization	no	no	DAB
162	CC1 Ventana, Benchmark	32 min	4B5	RTU	Ventana/Roche	F00396	32 min	UltraView Dab, Ventana	-	-	UltraView Dab, Ventana
164	CC1	36	4B5	predilute	Ventana	F00265	12	Ultraview	N	N	DAB
167	CC1	30 min	A0485	1/1700	DAKO	86782	32 min	Ultraview DAB kit	N	Cu	DAB
168	CC1	30 min	4B5	RTU	Roche Ventana	F00396	16	Ultraview DAB	N	Y	DAB
170	citrate ph 6.0 HIER	40 min	Her2	ready to use	Dako	200188177	30 min	Envision Flex Dako	no	no	DAB
175	HIER	36	4B5	Pre-dilute	Roche	F00396	16	Ultraview	N	Y	DAB
181	Ventana/Roche CC1	30 minutes	4B5	pre-diluted	Ventana/Roche	E10786	16 minutes	Ventana Ultraview DAB	no	yes	DAB
186	HIER	20 minutes	c-erbB-2 Polyclonal	1:400	Dako	20002512	15 minutes	Bond Polymer	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	F00265	24	Optiview	N	N	DAB
188	Bond Epitope Retrieval Solution 1	25	CB11	RTU	Leica	29662	30	Oracle HER2 IHC Detection System TA9145	Y	N	DAB
189	CC1	32	4B5	Pre-dilute	Roche/Ventana	unknown	16	Ultraview	N	N	DAB
190	CCI mild	?	SP3 rabbit monoclonal	1:50	Thermofisher	9103S1301A	40	IVIEW	N	N	DAB
191	CC1	30'	4B5	RTU	Roche	E10274	16'	ultraview DAB	N	N	DAB
194	CC1	30	4B5	predilute	VENTANA	F00396	12	IVIEW KIT(AVIDIN-BIOTIN)	N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Ventana/Roche	F00265	20 min	Ultraview	N	Y	DAB
199	HIER	25	CB11	RTU	Leica	29662	30	Leica Oracle Kit	N	N	DAB
202	Citrate PH 6.0	40 min	Her2	RTU	DAKO	20018877	30 min	Herceptest kit	n	n	dab
207	CC1	36 Minutes	4B5	Prediluted	Ventana	F02223	16 miutes	Ultraview DAB	N	N	DAB
215	CC1	36mins	4B5	Predilute	Roche	F00396	12	Ultraview	No	No	DAB
216	HIER	30	4B5	predilute	VENTANA/ROCHE	F00396	32	IVIEW KIT(AVIDIN-BIOTIN)	N	Y	DAB
217	CC1	32	4B5	pre-dilute	Roche-Ventana	E07785	20	Optiview	No	No	DAB
221	Visualizatin Solution	40	Hercep Test	NEAT	Dako	20016469	30	Hercep Visualization	N	N	DAB

Table S4. Descriptive statistics for ER based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	74	93.24	69	69/69 (100%)	1	1	1	1	1
102	74	93.24	69	64/69 (93%)	1	0.86	0.87	1	0.86
106	74	94.59	70	70/70 (100%)	1	1	1	1	1
107	74	95.95	71	71/71 (100%)	1	1	1	1	1
109	74	95.95	71	71/71 (100%)	1	1	1	1	1
111	74	95.95	71	71/71 (100%)	1	1	1	1	1
112	74	90.54	67	67/67 (100%)	1	1	1	1	1
113	74	95.95	71	70/71 (99%)	0.97	1	1	0.97	0.97
114	74	94.59	70	70/70 (100%)	1	1	1	1	1
115	74	94.59	70	69/70 (99%)	0.97	1	1	0.97	0.97
116	74	94.59	70	69/70 (99%)	0.97	1	1	0.97	0.97
119	74	93.24	69	68/69 (99%)	0.97	1	1	0.97	0.97
120	74	95.95	71	69/71 (97%)	0.94	1	1	0.95	0.94
122	74	94.59	70	70/70 (100%)	1	1	1	1	1
123	74	94.59	70	68/70 (97%)	0.94	1	1	0.95	0.94
124	74	95.95	71	69/71 (97%)	0.94	1	1	0.95	0.94
125	74	98.65	73	73/73 (100%)	1	1	1	1	1
126	74	97.3	72	72/72 (100%)	1	1	1	1	1
127	74	100	74	74/74 (100%)	1	1	1	1	1
129	74	94.59	70	70/70 (100%)	1	1	1	1	1
132	74	94.59	70	70/70 (100%)	1	1	1	1	1
133	74	97.3	72	72/72 (100%)	1	1	1	1	1
134	74	97.3	72	70/72 (97%)	0.94	1	1	0.95	0.94
135	74	94.59	70	66/70 (94%)	1	0.9	0.89	1	0.89
138	74	94.59	70	66/70 (94%)	0.88	1	1	0.9	0.89
139	74	95.95	71	67/71 (94%)	0.88	1	1	0.9	0.89
141	74	97.3	72	68/72 (94%)	0.88	1	1	0.91	0.89
143	74	98.65	73	73/73 (100%)	1	1	1	1	1
144	74	97.3	72	72/72 (100%)	1	1	1	1	1
145	74	95.95	71	71/71 (100%)	1	1	1	1	1
146	74	94.59	70	68/70 (97%)	0.94	1	1	0.95	0.94
147	74	100	74	74/74 (100%)	1	1	1	1	1
148	74	95.95	71	71/71 (100%)	1	1	1	1	1
149	74	98.65	73	69/73 (95%)	0.88	1	1	0.91	0.89
150	74	98.65	73	71/73 (97%)	0.94	1	1	0.95	0.94
151	74	97.3	72	72/72 (100%)	1	1	1	1	1
152	74	97.3	72	68/72 (94%)	0.88	1	1	0.9	0.89
153	74	98.65	73	73/73 (100%)	1	1	1	1	1
155	74	100	74	70/74 (95%)	0.88	1	1	0.91	0.89
157	74	98.65	73	73/73 (100%)	1	1	1	1	1
159	74	95.95	71	69/71 (97%)	0.94	1	1	0.95	0.94
160	74	98.65	73	69/73 (95%)	0.88	1	1	0.91	0.89
161	74	97.3	72	68/72 (94%)	0.88	1	1	0.91	0.89
162	74	98.65	73	73/73 (100%)	1	1	1	1	1
164	74	94.59	70	66/70 (94%)	0.88	1	1	0.9	0.88
165	74	97.3	72	72/72 (100%)	1	1	1	1	1
167	74	97.3	72	66/72 (92%)	0.82	1	1	0.87	0.83
168	74	94.59	70	67/70 (96%)	1	0.92	0.92	1	0.91
170	74	98.65	73	71/73 (97%)	0.94	1	1	0.95	0.94
173	74	94.59	70	68/70 (97%)	0.94	1	1	0.95	0.94
175	74	97.3	72	68/72 (94%)	0.88	1	1	0.91	0.89
177	74	94.59	70	66/70 (94%)	0.88	1	1	0.9	0.88
178	74	98.65	73	69/73 (95%)	0.88	1	1	0.91	0.89
180	74	95.95	71	71/71 (100%)	1	1	1	1	1
183	74	98.65	73	71/73 (97%)	0.94	1	1	0.95	0.94
184	74	94.59	70	68/70 (97%)	0.94	1	1	0.95	0.94
184a	74	95.95	71	69/71 (97%)	0.94	1	1	0.95	0.94
186	74	97.3	72	72/72 (100%)	1	1	1	1	1
187	74	94.59	70	68/70 (97%)	0.94	1	1	0.95	0.94
188	74	97.3	72	70/72 (97%)	0.94	1	1	0.95	0.94
189	74	95.95	71	71/71 (100%)	1	1	1	1	1
190	74	94.59	70	70/70 (100%)	1	1	1	1	1
191	74	98.65	73	73/73 (100%)	1	1	1	1	1
192	74	93.24	69	67/69 (97%)	0.94	1	1	0.95	0.94
194	74	74.32	55	55/55 (100%)	1	1	1	1	1
196	74	95.95	71	69/71 (97%)	0.94	1	1	0.95	0.94
198	74	89.19	66	66/66 (100%)	1	1	1	1	1
199	74	97.3	72	72/72 (100%)	1	1	1	1	1
202	74	93.24	69	60/69 (87%)	0.94	0.81	0.82	0.94	0.74
207	74	95.95	71	71/71 (100%)	1	1	1	1	1
208	74	94.59	70	63/70 (90%)	0.88	0.92	0.91	0.89	0.8
209	74	95.95	71	68/71 (96%)	0.9	1	1	0.93	0.91
215	74	97.3	72	70/72 (97%)	0.94	1	1	0.95	0.94
216	74	97.3	72	70/72 (97%)	0.94	1	1	0.95	0.94
217	74	94.59	70	67/70 (96%)	1	0.92	0.92	1	0.91
221	74	97.3	72	68/72 (94%)	0.88	1	1	0.91	0.89

Table S5. Descriptive statistics for PR based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	76	93.42	69	67/69 (97%)	0.9	1	1	0.96	0.93
102	76	94.74	70	69/70 (99%)	1	0.98	0.95	1	0.97
106	76	97.37	72	69/72 (96%)	1	0.94	0.88	1	0.9
107	76	98.68	73	70/73 (96%)	1	0.94	0.88	1	0.9
109	76	96.05	71	67/71 (94%)	1	0.92	0.83	1	0.87
111	76	89.47	66	64/66 (97%)	1	0.96	0.91	1	0.93
112	76	90.79	68	65/68 (96%)	0.86	1	1	0.94	0.89
113	76	96.05	72	67/72 (93%)	0.77	1	1	0.91	0.83
114	76	97.37	72	68/72 (94%)	1	0.92	0.84	1	0.87
115	76	92.11	69	67/69 (97%)	0.91	1	1	0.96	0.93
116	76	96.05	72	68/72 (94%)	0.82	1	1	0.93	0.86
119	76	94.74	70	68/70 (97%)	0.9	1	1	0.96	0.93
120	76	94.74	71	70/71 (99%)	0.95	1	1	0.98	0.97
122	76	96.05	72	69/72 (96%)	0.86	1	1	0.94	0.9
123	76	94.74	71	67/71 (94%)	0.82	1	1	0.92	0.86
124	76	94.74	71	69/71 (97%)	0.91	1	1	0.96	0.93
125	76	97.37	73	69/73 (95%)	0.82	1	1	0.93	0.86
126	76	98.68	73	68/73 (93%)	0.76	1	1	0.91	0.82
127	76	97.37	73	72/73 (99%)	0.95	1	1	0.98	0.97
129	76	98.68	73	69/73 (95%)	1	0.92	0.84	1	0.87
132	76	93.42	70	67/70 (96%)	0.86	1	1	0.94	0.9
133	76	100	74	73/74 (99%)	1	0.98	0.96	1	0.97
134	76	97.37	72	71/72 (99%)	1	0.98	0.95	1	0.97
135	76	93.42	69	65/69 (94%)	1	0.92	0.83	1	0.87
138	76	90.79	67	66/67 (99%)	1	0.98	0.95	1	0.97
139	76	96.05	72	68/72 (94%)	0.82	1	1	0.93	0.86
141	76	98.68	73	73/73 (100%)	1	1	1	1	1
143	76	98.68	73	69/73 (95%)	1	0.92	0.84	1	0.87
145	76	92.11	69	67/69 (97%)	0.91	1	1	0.96	0.93
146	76	94.74	70	65/70 (93%)	1	0.9	0.81	1	0.84
147	76	94.74	71	70/71 (99%)	0.95	1	1	0.98	0.97
149	76	94.74	71	69/71 (97%)	0.91	1	1	0.96	0.93
150	76	96.05	71	69/71 (97%)	0.95	0.98	0.95	0.98	0.93
151	76	96.05	72	69/72 (96%)	0.86	1	1	0.94	0.9
152	76	94.74	71	64/71 (90%)	0.68	1	1	0.88	0.75
153	76	98.68	73	68/73 (93%)	0.95	0.92	0.83	0.98	0.84
155	76	98.68	73	62/73 (85%)	0.95	0.8	0.68	0.98	0.68
157	76	96.05	72	70/72 (97%)	0.95	0.98	0.95	0.98	0.93
159	76	94.74	70	68/70 (97%)	1	0.96	0.91	1	0.93
160	76	93.42	69	67/69 (97%)	0.9	1	1	0.96	0.93
161	76	93.42	69	67/69 (97%)	0.95	0.98	0.95	0.98	0.93
162	76	97.37	72	69/72 (96%)	0.86	1	1	0.94	0.89
164	76	100	74	71/74 (96%)	0.86	1	1	0.95	0.9
165	76	96.05	71	61/71 (86%)	0.9	0.84	0.7	0.95	0.69
167	76	100	74	71/74 (96%)	0.86	1	1	0.95	0.9
168	76	100	74	71/74 (96%)	1	0.94	0.88	1	0.91
170	76	94.74	71	69/71 (97%)	0.91	1	1	0.96	0.93
173	76	97.37	73	66/73 (90%)	0.82	0.94	0.86	0.92	0.77
175	76	96.05	72	67/72 (93%)	0.77	1	1	0.91	0.83
177	76	96.05	72	64/72 (89%)	0.68	0.98	0.94	0.88	0.72
178	76	96.05	72	63/72 (88%)	0.59	1	1	0.85	0.67
183	76	98.68	73	64/73 (88%)	1	0.83	0.7	1	0.73
184	76	97.37	72	72/72 (100%)	1	1	1	1	1
184a	76	97.37	72	62/72 (86%)	0.95	0.82	0.69	0.98	0.7
186	76	97.37	72	66/72 (92%)	0.9	0.92	0.83	0.96	0.8
187	76	94.74	70	66/70 (94%)	0.95	0.94	0.87	0.98	0.87
188	76	96.05	71	67/71 (94%)	1	0.92	0.84	1	0.87
189	76	96.05	72	65/72 (90%)	1	0.86	0.75	1	0.79
190	76	97.37	72	70/72 (97%)	0.9	1	1	0.96	0.93
191	76	100	74	70/74 (95%)	0.95	0.94	0.88	0.98	0.87
192	76	94.74	71	65/71 (92%)	0.95	0.9	0.81	0.98	0.81
194	76	88.16	65	64/65 (98%)	0.95	1	1	0.98	0.96
196	76	96.05	71	71/71 (100%)	1	1	1	1	1
198	76	97.37	72	72/72 (100%)	1	1	1	1	1
199	76	98.68	74	70/74 (95%)	0.82	1	1	0.93	0.86
202	76	93.42	70	63/70 (90%)	0.82	0.94	0.86	0.92	0.77
207	76	98.68	73	70/73 (96%)	0.86	1	1	0.95	0.9
208	76	94.74	70	68/70 (97%)	1	0.96	0.91	1	0.93
209	76	94.74	70	67/70 (96%)	0.86	1	1	0.94	0.89
215	76	97.37	73	68/73 (93%)	0.95	0.92	0.84	0.98	0.84
216	76	97.37	72	70/72 (97%)	0.9	1	1	0.96	0.93
217	76	96.05	73	56/73 (77%)	1	0.67	0.56	1	0.55
221	76	96.05	72	68/72 (94%)	0.82	1	1	0.93	0.86

Table S6. Descriptive statistics for HER2 based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	75	96	72	72/72 (100%)	1	1	1	1	1
102	75	96	72	72/72 (100%)	1	1	1	1	1
106	75	93.33	70	70/70 (100%)	1	1	1	1	1
107	75	96	72	72/72 (100%)	1	1	1	1	1
109	75	98.67	74	74/74 (100%)	1	1	1	1	1
111	75	93.33	70	70/70 (100%)	1	1	1	1	1
112	75	89.33	67	67/67 (100%)	1	1	1	1	1
113	75	93.33	70	70/70 (100%)	1	1	1	1	1
114	75	97.33	73	73/73 (100%)	1	1	1	1	1
115	75	90.67	68	68/68 (100%)	1	1	1	1	1
116	75	90.67	68	68/68 (100%)	1	1	1	1	1
119	75	96	72	72/72 (100%)	1	1	1	1	1
120	75	98.67	74	74/74 (100%)	1	1	1	1	1
123	75	96	72	72/72 (100%)	1	1	1	1	1
124	75	93.33	70	70/70 (100%)	1	1	1	1	1
125	75	93.33	70	70/70 (100%)	1	1	1	1	1
126	75	98.67	74	74/74 (100%)	1	1	1	1	1
127	75	98.67	74	74/74 (100%)	1	1	1	1	1
129	75	98.67	74	74/74 (100%)	1	1	1	1	1
133	75	93.33	70	70/70 (100%)	1	1	1	1	1
135	75	96	72	72/72 (100%)	1	1	1	1	1
138	75	90.67	68	68/68 (100%)	1	1	1	1	1
139	75	97.33	73	73/73 (100%)	1	1	1	1	1
145	75	98.67	74	74/74 (100%)	1	1	1	1	1
147	75	100	75	75/75 (100%)	1	1	1	1	1
149	75	100	75	75/75 (100%)	1	1	1	1	1
150	75	98.67	74	74/74 (100%)	1	1	1	1	1
151	75	98.67	74	74/74 (100%)	1	1	1	1	1
152	75	98.67	74	73/74 (99%)	1	0.98	0.91	1	0.94
153	75	100	75	75/75 (100%)	1	1	1	1	1
155	75	98.67	74	74/74 (100%)	1	1	1	1	1
157	75	97.33	73	73/73 (100%)	1	1	1	1	1
160	75	96	72	72/72 (100%)	1	1	1	1	1
161	75	98.67	74	72/74 (97%)	0.8	1	1	0.97	0.87
162	75	94.67	71	71/71 (100%)	1	1	1	1	1
164	75	96	72	72/72 (100%)	1	1	1	1	1
167	75	98.67	74	73/74 (99%)	1	0.98	0.91	1	0.94
168	75	93.33	70	70/70 (100%)	1	1	1	1	1
170	75	97.33	73	73/73 (100%)	1	1	1	1	1
175	75	97.33	73	73/73 (100%)	1	1	1	1	1
181	75	97.33	73	73/73 (100%)	1	1	1	1	1
186	75	100	75	75/75 (100%)	1	1	1	1	1
187	75	97.33	73	73/73 (100%)	1	1	1	1	1
188	75	96	72	72/72 (100%)	1	1	1	1	1
189	75	96	72	72/72 (100%)	1	1	1	1	1
190	75	96	72	72/72 (100%)	1	1	1	1	1
191	75	94.67	71	71/71 (100%)	1	1	1	1	1
194	75	94.67	71	71/71 (100%)	1	1	1	1	1
198	75	97.33	73	73/73 (100%)	1	1	1	1	1
199	75	97.33	73	73/73 (100%)	1	1	1	1	1
202	75	94.67	71	71/71 (100%)	1	1	1	1	1
207	75	98.67	74	74/74 (100%)	1	1	1	1	1
215	75	97.33	73	73/73 (100%)	1	1	1	1	1
216	75	96	72	72/72 (100%)	1	1	1	1	1
217	75	98.67	74	74/74 (100%)	1	1	1	1	1
221	75	98.67	74	72/74 (97%)	1	0.97	0.82	1	0.88