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Assessors' report for cIQc Run 47: Breast Module (ER, PR and HER2)

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Assessment performed on Monday, June 24, 2015 at London Health Sciences Centre, London ON.

Background

Run 47 consisted of 40 cores, stained with the now familiar panel of ER, PR and HER2. Based on the self-assessment results it was anticipated that the overall results would be good and this was born out on review. General comments about each of the three markers follow, and then comments for individual laboratories, where the assessment team noted anything specific that they thought might be of help. As an overarching comment, one variable aspect of immunostaining that is readily apparent when doing a review of staining from many labs is the nuclear counter stain. A too-strong counterstain can make interpretation of weak nuclear immunoreactivity more challenging, and it is something to be aware of as it is easily adjusted.

ER

A trend has emerged in ER testing that all labs should be aware of, relating to weak ER positive cases (i.e. Allred scores 3-5). We have seen an increase in such cases with the introduction of the more sensitive polymer detection systems. This increase in weak positive cases is distinct from the issue identified simultaneously by cIQc, UKNEQAS and NordiQC around false positive results obtained by some laboratories using the combination of the 6F11 Ab and the Bond polymer detection kit, which we didn't see in this run, and consisted of a few labs seeing staining of cases that were completely negative in all other labs, and also negative by alternative testing methods e.g. mRNA. What is happening over the last few years is that there are clearly negative cases (e.g. cores 20 and 21), and clearly positive cases (e.g. cores 1-3), with the positive cases showing Allred 8/8 positivity. Then there are cases like cores 9, 16, 18, 22 and 27 and 30, with a few cells staining with some lab's protocols. A challenge with these cores is that only a few cells stain, and a negative result may reflect intratumoral heterogeneity and the small samples present in the 0.6 mm cores. What is the nature of these tumors? Four of them (cores 9, 16, 27 and 30) are HER2 amplified, and that is the dominant biomarker result. Core 16 shows weak PR positivity in many labs, suggesting that it is a true weak expresser of ER. Core 22 is negative for PR and HER2 in all labs, and in this case there would be significant variation in how it would be diagnosed i.e. weak ER positive versus triple negative.

It is important for everyone involved in breast cancer diagnosis and treatment to recognize that weak ER positivity (Allred scores 3-5) is not the same as the more common strong ER positivity (Allred scores 7 or 8), and such cases appear to be increasing with newer more sensitive detection systems. Most such cases are high grade or HER2 positive, and on that basis will be candidates for adjuvant chemotherapy. These cases with weak ER positivity include tumors that in the past would have been triple negative and recognized as being basal-like. They are still basal-like breast carcinomas, but are no longer ER negative with modern testing. We look forward to a publication from Dr. Torsten Nielsen's laboratory on this important development, whereby there has been an increasing percentage of basal-like breast cancers that show weak ER positivity, over recent years. We don't think that any action is required with respect to how we are performing ER testing, beyond being aware of this phenomenon.



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Apart from this general phenomenon, there was little noteworthy in the ER testing. We did change a significant number of results at review, often changing results to Unstatisfactory (no tumor) or changing Positive to Negative (staining in benign cells). In the weakly staining cores the assessment team tried to be consistent, but acknowledges the interpretive challenges associated with weak, focal positivity (is it less than or greater than 1%). Participant-specific feedback is summarized below:

Lab	IHC Status*	CIQC Comments
101	Optimal	
102	Optimal	
103	Optimal	Results not submitted online in time for the assessment meeting.
105	Optimal	
106	--	Slides not available
107	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
109	Optimal	
111	Optimal	
111a	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	
115	Optimal	
116	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
117	--	Slides not available
119	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
120	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
122	Optimal	
123	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
124	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
125	Optimal	
126	Adequate	Overall weaker staining than the other labs; protocol shows that the SP1 Ab is being used at 1:200, which is more dilute than is used elsewhere.
127	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
128	Optimal	
129	Optimal	



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132	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
133	Optimal	
134	Optimal	
135	Adequate	For some cores, intense nuclear staining led to cytoplasmic bleeding.
136	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
138	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
139	Optimal	
141	Optimal	
143	Optimal	
144	Optimal	
145	Optimal	
146	Optimal	
147	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
148	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
149	Optimal	
150	Optimal	
151	Optimal	
152	Optimal	
153	Optimal	
155	Optimal	
157	Optimal	
159	Sub-optimal	Possibly a staining artifact, but cores 1-4 were false negative, and it appears that the primary antibody did not get onto that area of the slide. The same result was observed for PR, from this lab. This most unusual result is difficult to understand; it may require a diagnostic check of their immunostainer to ensure that dispensing of primary antibody or deparaffinization are working properly.
160	Optimal	
161	Optimal	
162	Optimal	
164	Optimal	
165	Optimal	
167	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.



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168	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
170	Optimal	
173	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
175	Optimal	
177	--	Slides not available
178	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
180	Optimal	
183	Optimal	
184	Optimal	
184a	Optimal	
186	Optimal	
187	Optimal	
189	--	Slides not available
190	--	Slides not available
191	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.
194	Optimal	
196	Optimal	
198	Optimal	
199	Optimal	
202	Optimal	
207	--	Slides not available
208	Optimal	
209	--	Slides not available
215	Optimal	
216	Optimal	
217	Optimal	
221	Adequate	Slightly weaker staining than other labs, but this did not interfere with interpretation.

*Based on CIQC assessor consensus



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PR

Quality assurance/external proficiency testing for PR remains problematic, as there is no accepted “gold standard” for comparison. Using the consensus of results from labs across Canada remains the CIQC approach, and results on this basis were excellent. We are on the lookout for ER negative/PR positive cases, as this should, on a biological basis, be a vanishingly rare result; instead we expect to see a subset of ER positive cases showing PR positivity, which is precisely what was observed. A few false positive results (i.e. staining of cases that were consistently negative in other labs or, in the case of core 40, for example, was consistently ER negative AND negative for PR in other laboratories) were observed. 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
103	Satisfactory	Staining appeared satisfactory to cIQC assessors, but self-assessment results were not available online in time for the assessment meeting for comparison. Slight cytoplasmic background staining was observed.
135	Satisfactory	Moderate cytoplasmic and stromal background staining was observed.
159	Sub-optimal	The same curious problem of false negative/no staining of cores 1-4, as was noted above for the ER staining. These cores were HER2 negative so we are not able to comment on whether the same phenomenon also happened with the HER2 slide.
164	Satisfactory	False positive staining on cores 30 and 40
175	Satisfactory	False positive staining on cores 37 and 38

*Based on cIQC assessor consensus

HER2

Negative results for some cores were changed to uninterpretable/unsatisfactory (lab 129, core 21; lab 157, core 33; lab 170, core 38). The data entry for lab 120, where the data appeared to have been shifted by one space, was extensively revised and after this adjustment the results were consistent with all other participants. ***After assessment review, there were no false positive or false negative results in Canada and our few international participants (based on more than 2000 data points)!!! This is an extraordinary result. Congratulations!***

Garrattograms 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. Please note that cores with variable results across labs (due to true sampling heterogeneity) and cores with unsatisfactory results for more than 50% of labs were excluded from analysis. 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 #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	SP1	1:50	Lab Vision	9101S1406E	32 minutes	OptiView	N	Y	DAB
102	Dako 3 in 1 High pH	10/20/10	SP1	1:35	Thermo (Labvision)	9101S1406K	30' RT	DAKO FLEX	NO	CUSO4	DAB+
105	CC1 standard	60min	SP1	1:50	Thermo Scientific	9101S1210M	1 hour	Ultramap anti-Rb HRP	N	N	DAB
106	microwave pressure cooker	3 minutes	6F11	1:120	Leica	6027943	60 minutes	avidin boitin	no	no	DAB
107	ultra cc1	36 min	SP1	Predilute	Ventana	E07165	16 min	ultraview DAB	N	Y	DAB
109	HIER CC1	64 MIN	SP1	RTU	VENTANA	E06774	32 MIN	ULTRAVIEW DAB	N	Y	DAB
111	CC1	36 min	SP1	Predilute	Ventana	E04942	32 min	Ultraview	N	Copper	DAB
111a	Same as 111										
112	BOND ER 2 pH 9.0	20	SP1	1:150	ThermoFisher	PI1914231	15	BOND POLYMER REFINE	NO	NO	DAB
113	CC1	32'	SP1	1/70	ThermoScientific	9101S1406H	30'	Optiview	N	Y	DAB
114	CC1	32	SP1	1:50	THERMO FISHER	9101S1406K	16	OPTIVIEW	N	Y	DAB
115	CC1	30min	SP1	Predilute	Ventana	E06774	32	IVIEW DAB	Y	Cooper	DAB
116	CC1	64 min	SP1	RTU	VENATANA ROCHE	E07165	32 MIN	ultraView DAB	no	.	DAB
117	CC1	38 min	SP1	RTU	Ventana	E08617	32 min	Ultraview	N	CUSO4	DAB
119	HIER/CC1	-	SP1	Predilute	Ventana	-	-	Ultraview	-	N	DAB
120	waterbath	20	EP1	Predilute	Dako	10095094 exp 2015-11	20	Autostainer Link 48	N	N	Dab
122	HIER	20min ER2	SP1	RTU	Ventana	E02453	20	DAB	N	N	DAB
123	CC1	30	SP1	Predilute	Ventana/ Roche	E04942	32 min	Ultraview	N	Copper	DAB
124	CC1	60	SP1	1/100	Cell marque	1422401B	32	Dab Iview	n	n	DAB
125	Bond ER2	20 min	SP1	Predilute	Roche	E09654	15 min	Bond Polymer Refine	N	Y	DAB
126	Steamer	20 mins	SP1	1:200	ThermoFisher/Lab Vision (Neomarkers)	9101s1406k	30 mins	Envision	No	No	DAB
127	Automated Ventana Ultra CC1	36 Minutes	SP1	Predilute	Ventana	E02456	32 Minutes	Ventana Ultra Iview	N	N	DAB
128	HIER	64	SP1	RTU	VENTANA	E06774	16	ULTRAVIEW DAB	N	Y	N
129	ER2 - high pH retrieval	20 min	SP1	1:50	Thermo Scientific	9010S1308H	15 min	Leica Bond Refine Kit	N	N	DAB
132	Flex High pH	45	EP1	RTU	Dako	10097676	30	Flex	N	N	DAB
133	HIER	36 minutes	SP1	Predilute	Roche	E02456	24 minutes	Ultraview	no	no	dab
134	HIER	30	SP1	RTU	Roche	E02456	8	Ventana Ultraview	N	N	DAB
135	buffer PH 9	30 min	SP1	1:50	Thermal fisher	1210L	15 min	BOND DAB refined kit	N	N	DAB
136	HIER LOW	40	EP1	RTU	DAKO	10097676	20	POLYMER	n/a	No	DAB
138	HIER pH 9	20	EP1	RTU	Dako	10095666	20	Polymer	N	N	DAB
139	hier	30	SP1	RTU	Ventana	E10753	32	Iview	N	N	dab
141	HIER	30 min	SP1	RTU	Ventana/Roche	E02456	8 min	ultraView	N	N	DAB
143	HIER, automated on-line system	>45 but <60 min	SP1	no	Confirm Ventana	E06774	32 min	I view DAB detection kit	no	no	no
144	CC1	24 mn	SP1	1:50	ThermoScientific	9101S1406G	16 min	Optiview	No	Yes, copper	DAB
145	CC1	32	SP1	1/100	CELL MARQUE	1316403E	28	XT OPTIVIEW ihc v4	NO	NO	DAB
146	Flex TRS High	20	EP1	RTU	Dako	10087383	20	EnVision Flex Peroxydase	n	n	DAB
147	pH9, EDTA (ER2)	20	SP1	1:150	Thermo Fisher Scientific	9101S1406G	15	Bond Refine DAB kit	N	N	DAB
148	cc1	36	SP1	RTU	Ventana	E08617	8	Ultraview DAB			
149	PT Link high pH 97 C	20	EP1	RTU	Dako	10095094	20	EnVision Flex	N	N	DAB
150	cc1	30	sp1	n/a	ventana	0	16	ultraview	n	y	dab
151	HEIR 2 BUFFER pH9.0	20 MIN	SP1	1:50	THERMO SCIENTIFIC	9101S1406H	15 MIN	BOND REFINE POLYMER	N	N	DAB
152	HIER (ph > 7)	60	SP1	Predilute	Roche Ventana	E02456	32	iVIEW	N	Y	DAB
153	HIER CC1	32	SP1	Predilute	Ventana	E06774	8	Ultraview Dab	N	Y	DAB
155	CC1	30	SP1	Predilute	Ventana	E06774	40	Ultraview	N	Y	Dab
157	CC1	24 MIN.	SP 1	Predilute	VENTANA	E 02453	24 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
159	High pH	40 total	EP1	Prediluted	DAKO	10095666	20	FLEX	No	No	DAB
160	CC1	36 MIN	SP1	Predilute	VENTANA	E07165	8 MIN	ULTRAVIEW	N	CUSO4	DAB
161	HIER-High EDTA TRIS buffer	20 minutes	EP1	RTU	DAKO	10097676	20 minutes	Envision Flex	no	no	DAB
162	CC1 (ventana)	48	SP1	1:100	Thermo Scientific	9101s1406G	32 min	Optiview (ventana)	N	N	DAB (ventana)
164	ultraCC1	36	SP1	Predilute	Ventana	E08617	8	UltraView	N	N	DAB
165	hier	30 min	sp1	nil	ventana	e06774	28 min	ultraview	n	n	dab
167	CC1	30 min	SP1	Predilute	Roche	D08925	8 min	Ultraview DAB kit	N	Y (copper)	DAB
168	Heat Induced Epitope Retrieval	48 min	SP1	1/100	Cellmarque	1422401B	30 min	Envision Flex +	N	Y	DAB
170	HIER	20 min	Ep1	RTU	Dako	10095094	20 min	EnVision Flex Dako	no	no	DAB
173	HIER	30	Sp1	Predilute	Ventana	E03158	16	Ultraview-Polymer	N	Y	DAB
175	HIER	36	SP1	Predilute	Roche	E08617	32	polymer (ultraview)	N	Y-copper	dab
177	CC!	30 min	6F11	1 : 25	Novocastra		32 min	Ultraview	y	y	DAB
178	HIER	32	SP1	N/A	Ventana	E08617	16	Ultraview	N	N	DAB
180	Ventana CC1	32 min	SP1	Predilute	Ventana	E00035	16 min	Optiview	N	N	DAB
183	ultra cc1	36 min	SP1	Predilute	Roche	E04936	32	ultraview	n	n	dab
184	Pascal with Target Retrieval Solution	5 min at 125 C, cool to 90 C	1D5 and ER-2-123	RTU	Agilent (Dako)	10096512	30	SK310, ER/PR pharm Dx Kit	N	N	DAB+
184a	Hi pH Target Retrieval Solution	20	EP1	RTU	Agilent (Dako)	10097676	20	FLEX	N	N	DAB+
186	HIER EDTA buffer PH9	20 min	SP1	1:50	Thermo scientific RM-9101S	9101S1308B	15 min	Bond Refine	N	N	DAB
187	CC1	16	SP1	Predilute	Roche	E08617	8	Optiview	None	None	DAB
189	Ventana CC1	64 min.	SP1	Predilute	Ventana	unknown	16 min.	Ultraview	N	N	Ultraview DAB
190	Ventana CC1	32 min	SP1	Predilute	Ventana	E08617	32 min	Ventana IView DAB	N	N	DAB
191	CC1	30'	SP1	RTU	Roche	E02456	16'	ultraview DAB	N	N	DAB
194	CC1	30	SP1	Predilute	VENTANA	E07165	12	IVIEW KIT(AVIDIN-BIOTIN)	Y	Y	DAB
196			SP1		Ventana	E08617	8.0 min.	DAB			
198	Citrate pH 6.2 pressure cooker in microwave	5 min	6F11	1/100	Novocastra/Leica	6027943	30 min RT	MACH 1 polymer	N	Y	DAB
199	Bond ER-1 (Heat)	20	6F11	Bond RTU (PA0151)	Leica Biosystems	28707	20	Polymer (Refine DS9800)	N	N	DAB
202	BOND ER2 solution pH9.5	20 min	6F11	1/300	Vector	6018294	15 MIN RT	Leica Refine Detection kit	N	N	dab
207	heat-CC1	36minutes	SP1	Prediluted	Roche	e07165	16 minutes	Ultra View DAB	N	N	DAB
208	HIER	30	6F11	1/50	LEICA	6027943	60	Envision Flex	N	N	DAB Flex
209	HIER	20 mins at 97C and 20mins to cool to 85C	EP1	Predilute	Dako	10097676	20mins	Envision plus	N	N	DAB
215	CC1	64	SP1	Predilute	Roche/Ventana	E02453	32	Ultraview	N	N	DAB
216	HIER	30	SP1	Predilute	Ventana/Roche	E08617	24	Avidin/Biotin (Iview Kit)	N	Y	DAB
217	Benchmark Ultra CC1 100 degrees C	64 min	SP1	Predilute	Ventana	E06774	20 min at 36 degrees C	Ventana Optiview 760-700	no	CUSO4	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 Lot #	Ab Supplier/Vendor	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	Clone 16	1:100	6015355	Leica	32 minutes	OptiView	N	Y	DAB
102	DAKO 3 in 1 High pH	10/20/10	16	1:150	6027462	Leica	30' RT	DAKO FLEX	NO	CUSO4	DAB+
105	CC1 standard	60 min	PgR636	1:200	100885019	Dako	60 min	Ultramap anti Ms HRP	N	Y	DAB
106	microwave pressure cooker	3 minutes	PgR 1294	1:1500	10070965	Dako	60 minutes	avidin biotin	no	no	DAB
107	ultra cc1	64 min	PgR 1294	1:50	Dako	10092055	32 min	ultraview DAB	Y	Y	DAB
109	HIER CC1-HIGH pH	36 MIN	1294	1/50	10082389	DAKO	16 MIN	ULTRAVIEW DAB	N	Y	DAB
111	CC1	48 min/RT	16	1/80	6022579	Leica	32 min	Optiview	N	Copper	DAB
111a	CC1	36	16	Predilute	6027464	Ventana	32	Ultraview	N	CUSO4	DAB
112	BOND ER2 pH 9.0	12	16	RTU	25509	LEICA	15	BOND POLYMER REFINE	NO	NO	DAB
113	Low pH	30'	Pgr 636	1/300	10090701	Dako	20'	Flex	Y	N	DAB
114	CC1	32	16	1:25	6027462	NOVOCASTRA	16	OPTIVIEW	N	Y	DAB
115	CC1	30min	IE2	Predilute	E07088	Ventana	32	IVIEW DAB	Y	Cooper	DAB
116	CC1	64 MIN	CLONE 16	1/100	27462	LEICA	48 MIN	ultraView DAB	yes	.	DAB
117	CC1	38 min.	PR(16)	1/200	6027462	Leica	12 min	Ultraview	N	CUSO4	DAD
119	HIER/CC1	-	16	-	--	Ventana	-	Ultraview	--	N	DAB
120	waterbath	20	PgR 636	Predilute	10083456	Dako	20	Autostainer Link 48	N	N	DAB
122	HIER	20min ER2	16	ready to use	27430	Leica Nova Castra	15 min	polymer refined	N	N	DAB
123	CC1	60	16	1/50	6031757	Vector Laboratories	32	Ultraview	N	Copper	DAB
124	CC1	30	IE2	Predilute	E07088	Ventana	20	DAB Iview	n	n	Dab
125	Bond ER2	20 min	16	Predilute	27787	Leica	15 min	Bond Polymer Refine	N	Y	DAB
126	Steamer	20 mins	PgR 636	1:500	10090531	DAKO	30 mins	Envision	No	No	DAB
127	Automated Ventana Ultra CC1	36 Minutes	IE2	Predilute	D09110	Ventana	8 Minutes	Ultra Iview	N	N	Dab
128	HIER	64	IE2	NONE	E07088	VENTANA	16	ULTRAVIEW DAB	N	Y	N
129	ER2 - high pH retrieval	20 min	16	1:400	Z050510	Novocastra	15 min	Leica Bond Refine Kit	N	N	DAB
132	Flex High pH	45	PR16	1/200	6024555	Vector	30	Flex	N	N	DAB
133	HIER	64 minutes	16	1/50	6024555	Vector	32 minutes	Ultraview	no	no	dab
134	HIER	30	IE2	RTU	E02706	Roche	12	Ventana Ultraview	N	N	DAB
135	buffer PH 9	20 min	clone 16	1:400	1312100	Leica	15 min	BOND DAB refined kit	N	N	DAB
136	HIER LOW	40	PGR636	RTU	10096659	DAKO	20	POLYMER	n/a	No	DAB
138	HIER pH 9	20	PgR 636	RTU	10096659	Dako	20	Polymer	N	N	DAB
139	hier	30	IE2	ready use	E09558	Ventana	32	Iview	N	N	dab
141	HIER	30 min	IE2	Ready to use	E02706	Ventana/Roche	12 min	ultraView	N	N	DAB
143	HIER automated on-line system	>45 but <60 min	IE2	no	E02707	Confirm Ventana	32 min	I View DAB detection kit	n	y	no
145	CC1	32	16	1/100	6027462	NOVOCASTRA	28	XT OPTIVIEW ihc v4	NO	NO	DAB
146	FLES TRS HIGH	20	636	RTU	10081639	Dako	30	EnVision Flex Peroxydase	n	n	DAB
147	pH9, EDTA (ER2)	20	16	1:800	6027295	NCL	15	Bond Refine DAB kit	N	N	DAB
149	PT Link high pH 97 C	20	PGR 636	RTU	10090679	Dako	20	EnVision Flex	Y	N	DAB
150	cc1	30	IE2	n/a	0	Ventana	20	ultraview	n	y	dab
151	HIER 1 BUFFER pH6.0	20 MIN	16	1:200	6027295	NCL	15 MIN	BOND REFINE POLYMER	N	N	DAB
152	HIER (pH > 7)	60	IE2	Pre-diluted	E04627	Roche Ventana	32	iVIEW	N	Y	DAB
153	HIER CC1	32	IE2	Predilute	E07088	Ventana	24	Ultraview Dab	N	Y	DAB
155	CC1	30	IE2	Predilute	E07088	Ventana	32	ultraview	n	y	dab
157	CC1	24 MIN.	IE 2	Predilute	E 02707	VENTANA	24 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
159	High pH	40 total	PgR 636	Prediluted	10093047	DAKO	30	FLEX	No	No	DAB
160	CC1	36 MIN	IE2	Predilute	E06575	VENTANA	8 MIN	ULTRAVIEW	N	CUSO4	DAB
161	HIER-High EDTA TRIS buffer	20 minutes	PgR636	RTU	10096659	DAKO	20 minutes	Envision Flex	no	mouse linker	DAB
162	CC1 (ventana)	48 min	16	1:80	6020162	Dako	32 min	OptiView (ventana)	N	N	DAB
164	ultraCC1	36	IE2	Predilute	E09558	Ventana	8min	UltraView	N	N	DAB
165	hier	30 min	IE2	nil	e07088	ventana	24 min	ultraview	n	n	dab
167	CC1	30 min	IE2	Predilute	D09110	Roche	8 min	Ultraview DAB kit	N	CUSO4	DAB
168	Heat Induced Epitope Retrieval	48 min	PgR636	RTU	10096659	Dako	20 min	Envision Flex +	N	Y	DAB
170	HIER	20 min	PgR636	ready to use	10095511	Dako	20 min	EnVision Flex Dako	no	no	DAB
173	HIER	30	IE2	Predilute	D09634	Ventana	16	Ultraview-Polymer	N	Y	DAB
175	HIER	64	IE2	Predilute	E07088	Roche	32	polymer (ultraview)	N	CUSO4	Dab
177	CC1	32	Pgr 636	1 : 25		Dako	32	Ultraview	y	y	DAB
178	HIER	32	IE2	N/A	E02712	Ventana	16	Ultraview	N	N	DAB
183	ultra cc1	36 min	IE2	predilute	E02707	roche	32 min	ultraview	n	n	dab
184	Pascal with Target Retrieval soln	5 min at 125 C, cool to 90 C	PGR 1294	RTU	10096512	Agilent (Dako)	30	SK310, ER/PR pharm Dx kit	N	N	DAB+
184a	Hi pH Target Retrieval	20	PgR 636	RTU	10098331	Agilent (Dako)	20	FLEX+	N	N	DAB+
186	HIER EDTA BUFFER PH9	20 min	PR88	1:100	MJ3281213	Biogenex	15 min	Bond refine detection system	N	N	DAB
187	CC1	64.0	IE2	Predilute	E07088	Roche	12	Ultraview	None	None	DAB
189	Ventana CC1	64 min.	IE2	Predilute	unknown	Ventana	16 min.	Ultraview	N	N	Ultraview DAB
190	Ventana CC1	32 min	Clone 16	1:50	6015355	Novocastra (Leica)	32 min	Ventana IView DAB	N	N	DAB
191	CC1	30'	IE2	RTU	D07381	Roche	16'	ultraview DAB	N	N	DAB
194	CC1	30	IE2	Predilute	E02706	VENTANA	20	IVIEW KIT(AVIDIN-BIOTIN)	N	Y	DAB
196			IE2		E04944	VENTANA	8.0 min	DAB			
198	Citrate pH 6.2 pressure cooker in microwave	5 min	16	1/600	6024555	Vector Laboratories	30 min RT	MACH 1 polymer	N	Y	DAB
199	Bond ER-2 (Heat)	20	16	1:200	6027462	Leica Biosystems	15	Polymer (Refine D9S900)	N	N	DAB
202	Bond ER2 solution pH 9.5	30 min	16	RTU	29086	LEICA	15 MIN RT	LEICA REFINE DETECTION KIT	N	N	DAB
207	On line heat-CC1	36minutes	IE2	Predilute	E04627	Roche	16 minutes	Ultra View DAB	N	N	DAB
208	HIER	30	PGR636	Predilute	10095092	DAKO	60	ENVISION FLEX	N	N	DAB FLEX
209	HIER	20 mins at 97C, 20 mins cooling to 85C	PgR 636	Predilute	10095092	Dako	20mins	Envision plus	N	N	DAB
215	CC1	64	IE2	Predilute	E04944	Roche/Ventana	16	Ultraview	N	N	DAB
216	HIER	30	IE2	Predilute	E07088	Ventana/Roche	24	Avidin/Biotin (IView Kit)	N	Y	DAB
217	HIER Benchmark Ultra CC1 100 degrees C	64 min	IE2	Predilute	E07088	Ventana	16 min at 36 degrees C	Ventana Optiview 760-700	no	CUSO4	DAB
221	pH6 Citrate Buffer	20	1A6	1:200	6027462	Leica	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 #	Time for Ab Dilution (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	SP3	1:200	Lab Vision	9103S1305I	32 min	OptiView	N	Y	DAB
102	Dako 3 in 1 High pH	10/20/10	SP3	1:750	Thermo (Labvision)	9103S1306P	30' RT	Dako FLEX	NO	CUSO4	DAB+
105	CC1 standard	60min	4B5	Neat	Ventana	D08847	32min	DABMAP	N	N	DAB
106	HIER Automated Stainer	36	4B5	Predilute	Ventana	E08669	16 min	ultaview universal DAB detection kit	no	no	DAB
107	ultra cc1	36 min	4B5	Predilute	Ventana	E07785	8 min	ultraview DAB	N	Y	DAB
109	HIER -CC1 high pH	36 MIN	4B5	RTU	VENTANA	E09042	16 MIN	ULTRAVIEW DAB	N	Y	DAB
111	CC1	36 min	4B5	Predilute	Ventana	E10786	32 min	Ultraview	N	Copper	DAB
111a	Same as 111										
112	BOND ER2 pH 9.0	20	4B5	1:3 RATIO of the RTU	VENTANA	E00842	15	BOND POLYMER REFINE	NO	NO	DAB
113	CC1	32'	SP3	1/50	Esbe/CellMarque	1332302B	16'	Ultraview	N	Y	DAB
114	CC1	32	SP3	1:200	THERMO FISHER	9103S1202E	16	OPTIVIEW	N	Y	DAB
115	CC1	30min	4B5	Predilute	Ventana	E08669	24 (incubation)	IVIEW DAB	Y	Cooper	DAB
116	CC1	40 MIN	SP3	1/100	THERMO FISHER	1306 F	36 MIN	Optiview DAB IHC	no	-	DAB
117	CC1	38min.	SP3	1/200	Ventana	9103511305I	32min.	ultraview	nN	CUSO4	Dab
119	HIER/CC1	-	4B5	-	Ventana	-	-	Ultraview	-	N	DAB
120	Waterbath	40	HER2 PROTEIN	Predilute	Dako	20016932	40	Autostainer Link 48	N	N	DAB
123	CC1	30 min	4B5	Predilute	Ventana/ Roche	E10786	32 min	Ultraview DAB	N	Copper	DAB
124	CC1	30	4B5	Predilute	Ventana	E07785	12	Dab Iview	n	n	DAB
125	Ventana CC1 standard	36 min	4B5	Predilute	Roche	E08669	16 min	Ventana Ultraview	N	N	DAB
126	Steamer	30 mins	SP3	1:400	DAKO	9103s1407c	30 mins	Envision	No	No	DAB
127	Automated Ventana ultra CC1	36 Minutes	4B5	Predilute	Ventana	E09042	24 Minutes incubation	Ventana Ultra iview	N	N	DAB
129	ER1 - low pH retrieval	20 minutes	Cerb b2/ A0485	1:600	Dako	86782	15min	Leica Bond Refine Kit	N	N	DAB
133	HIER	36 minutes	4B5	Predilute	Roche	E07785	24 minutes	Ultraview	no	no	dab
135	buffer PH 6	20 min	A0485	1:700	Dako	81393	15 min Ab incubation	BOND DAB refined kit	N	N	DAB
136	HIER	60	A0485	RTU	Dako	20018782	30	POLYMER	n/a	No	DAB
138	HIER pH 6	20	A0485	1:600	Dako	20019646	30	Polymer	N	N	DAB
139	HIER	30	4B5	ready use	Ventana	E10786	32	iview dab	N	N	DAB
145	CC1	32	SP3	1/300	CELL MARQUE	1332302A	16	XT OPTIVIEW ihc v4	NO	NO	DAB
147	pH6, Citrate (ER1)	20	Polyclonal	1:400	Dako	92075	15	Bond Refine DAB kit	N	N	DAB
149	PT Link low pH 97 C	20	A0485	1:500	Dako	20012899	20	EnVision Flex	y	N	DAB
150	cc1	30	sp3	1/100	Neomarkers	0	20	ultraview	n	y	dab
151	HEIR 1 BUFFER pH 6.0	20 MIN	POLYCLONAL	1:500	DAKO	20002512A	15 MIN	BOND REFINE POLYMER	N	N	DAB
152	HIER (pH > 7)	60	4B5	Predilute	Roche Ventana	E09042	32	iVIEW	N	Y	DAB
153	hier CC1	32	4B5	Predilute	Ventana	E08669	32	Ultraview Dab	N	Y	DAB
155	CC1	30	4B5	Predilute	Ventana	E08669	32	Ultraview	n	Y	DAB
157	CC1 24 MIN.	24 MIN	4B 5	Predilute	VENTANA	E 04951	24 MIN.	BENCHMARK XT	Y	Y	OPTIVIEW
160	CC1	36 MIN	POLYCLONAL	1/700	DAKO	20012899	32 MIN	ULTRAVIEW	N	CUSO4	DAB
161	Herceptest epitope retrieval solution	40 minutes	Rabbit anti-human	RTU	DAKO	20016932	30 minutes	Herceptest visualization reagent	no	no	Herceptest DAB
162	CC1 (ventana)	32 min	4B5	RTU	Ventana	E09042	20 min	ultraView (ventana)	n	N	DAB (ventana)
164	ultra CC1	36	4B5	Predilute	Ventana	E10786	12	UltraView	N	N	DAB
167	CC1	30 min	A0485	1/1700	DAKO	86782	32 min	Ultraview DAB kit	N	Y (copper)	DAB
168	Cell conditioning	30 min	4B5	RTU	Ventana	E10257	16 min	Ultraview DAB	N	Y	DAB
170	HIER	40 min	ERB-2	ready to use	Dako	20013072	30 min	EnVision Flex Dako	no	no	DAB
175	HIER	32	4B5	Predilute	Roche	E10257	16	polymer	N	Y-copper	Dab
181	Ventana/Roche CC1	30 minutes	4B5	Predilute	Ventana/Roche	E10786	16 minutes	Ventana Ultraview DAB	no	yes	DAB
186	HIER citrate buffer pH6	20 min	polyclonal	1:400	DAKO	20002512	15 min	Bond Refine Detection System	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	E10786	24	Optiview	None	None	DAB
189	Ventana CC1	32 min.	4B5	Predilute	Ventana	unknown	16 min.	Ultraview	N	N	Ultraview DAB
190	Ventana CC1	32 min	SP3	1:50	ThermoFisher	9103S1306F	40 min	Ventana IView DAB	Y	N	DAB
191	CC1	30'	4B5	RTU	Roche	E06148	16'	ultraview DAB	N	N	DAB
194	CC1	30	4B5	Predilute	VENTANA	E08669	12	IVIEW KIT(AVIDIN-BIOTIN)	N	Y	DAB
198	CC1	36 min	4B5	Predilute	Ventana/Roche	E07785	20 min	Ultraview	N	Y	DAB
199	Bond ER-2 (Heat)	20	CB11	Oracle Kit - RTU (TA9145)	Leica Biosystems	27595	0	Polymer (Oracle Kit)	None	N	DAB
202	citrate buffer 6.0	40 MIN	Her2	RTU	DAKO Herceptest	20018799	30 min	Dako Herceptest	no	no	DAB
207	on line heat-CC1	36 minutes	4B5	Prediluted	Roche	E09042	16 minutes	UltraView DAB	N	N	DAB
215	CC1	36	4B5	Predilute	Roche/Ventana	E04951	12	DAB	N	N	DAB
216	HIER	30	4B5	Predilute	Ventana/Roche	E08669	32	Avidin/Biotin (IView Kit)	N	Y	DAB
217	HIER Benchmark Ultra CC1	32 minutes	4B5	Predilute	Ventana	E06774	20 minutes at 37 degrees C	Ventana Optiview 760-700	NO	CUSO4	DAB
221	Hercep Test Epitope Retrieval Solution	40	Hercep Test	NEAT	Dako	20015605	30	Dako Visualization Reagent	Y	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	31	100	31	29/31 (94%)	0.95	0.9	0.95	0.9	0.85
102	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
103	31	100	31	29/31 (94%)	0.95	0.9	0.95	0.9	0.85
105	31	77.42	24	24/24 (100%)	1	1	1	1	1
106	31	100	31	29/31 (94%)	0.95	0.9	0.95	0.9	0.85
107	31	77.42	24	23/24 (96%)	0.94	1	1	0.86	0.89
109	31	93.55	29	28/29 (97%)	0.95	1	1	0.89	0.92
111	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
111a	31	83.87	26	25/26 (96%)	0.94	1	1	0.89	0.91
112	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
113	31	77.42	24	23/24 (96%)	0.94	1	1	0.86	0.89
114	31	100	31	29/31 (94%)	0.95	0.9	0.95	0.9	0.85
115	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
116	31	93.55	29	28/29 (97%)	0.95	1	1	0.89	0.92
117	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
119	31	83.87	26	25/26 (96%)	0.94	1	1	0.89	0.91
120	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
122	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
123	31	96.77	30	29/30 (97%)	0.95	1	1	0.91	0.93
124	31	83.87	26	25/26 (96%)	0.94	1	1	0.9	0.92
125	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
126	31	83.87	26	25/26 (96%)	0.94	1	1	0.89	0.91
127	31	70.97	22	21/22 (95%)	0.94	1	1	0.83	0.88
128	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
129	31	96.77	30	29/30 (97%)	0.95	1	1	0.91	0.93
132	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
133	31	74.19	23	22/23 (96%)	0.94	1	1	0.86	0.89
134	31	93.55	29	28/29 (97%)	0.95	1	1	0.91	0.93
135	31	100	31	28/31 (90%)	0.95	0.8	0.91	0.89	0.77
136	31	90.32	28	27/28 (96%)	0.94	1	1	0.91	0.92
138	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
139	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
141	31	90.32	28	27/28 (96%)	0.95	1	1	0.89	0.92
143	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
144	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
145	31	93.55	29	27/29 (93%)	0.95	0.89	0.95	0.89	0.84
146	31	96.77	30	28/30 (93%)	0.95	0.9	0.95	0.9	0.85
147	31	80.65	25	24/25 (96%)	0.94	1	1	0.88	0.9
148	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
149	31	93.55	29	28/29 (97%)	0.95	1	1	0.91	0.93
150	31	70.97	22	21/22 (95%)	0.94	1	1	0.83	0.88
151	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
152	31	96.77	30	29/30 (97%)	0.95	1	1	0.91	0.93
153	31	93.55	29	28/29 (97%)	0.95	1	1	0.91	0.93
155	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
157	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
159	31	93.55	29	23/29 (79%)	0.79	0.8	0.88	0.67	0.56
160	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
161	31	96.77	30	29/30 (97%)	0.95	1	1	0.91	0.93
162	31	100	31	29/31 (94%)	0.95	0.9	0.95	0.9	0.85
164	31	90.32	28	27/28 (96%)	0.95	1	1	0.9	0.92
165	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
167	31	96.77	30	29/30 (97%)	0.95	1	1	0.91	0.93
168	31	90.32	28	27/28 (96%)	0.95	1	1	0.9	0.92
170	31	90.32	28	27/28 (96%)	0.95	1	1	0.9	0.92
173	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
175	31	74.19	23	22/23 (96%)	0.94	1	1	0.86	0.89
177	31	100	31	29/31 (94%)	0.9	1	1	0.83	0.86
178	31	90.32	28	26/28 (93%)	0.89	1	1	0.82	0.85
180	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
183	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
184	31	83.87	26	25/26 (96%)	0.94	1	1	0.89	0.91
184a	31	80.65	25	24/25 (96%)	1	0.88	0.94	1	0.9
186	31	80.65	25	24/25 (96%)	0.94	1	1	0.89	0.91
187	31	87.1	27	26/27 (96%)	0.94	1	1	0.91	0.92
189	31	80.65	25	24/25 (96%)	1	0.88	0.94	1	0.9
190	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
191	31	80.65	25	23/25 (92%)	0.94	0.88	0.94	0.88	0.82
194	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
196	31	93.55	29	28/29 (97%)	0.95	1	1	0.9	0.92
198	31	83.87	26	24/26 (92%)	0.94	0.88	0.94	0.88	0.82
199	31	96.77	30	29/30 (97%)	0.95	1	1	0.9	0.92
202	31	93.55	29	28/29 (97%)	0.95	1	1	0.9	0.92
207	31	83.87	26	24/26 (92%)	0.94	0.88	0.94	0.88	0.82
208	31	100	31	30/31 (97%)	0.95	1	1	0.91	0.93
209	31	90.32	28	27/28 (96%)	0.95	1	1	0.9	0.92
215	31	96.77	30	28/30 (93%)	0.95	0.89	0.95	0.89	0.84
216	31	83.87	26	25/26 (96%)	0.94	1	1	0.89	0.91
217	31	100	31	29/31 (94%)	1	0.8	0.91	1	0.84
221	31	87.1	27	26/27 (96%)	0.95	1	1	0.89	0.91

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	29	96.55	28	28/28 (100%)	1	1	1	1	1
102	29	79.31	23	23/23 (100%)	1	1	1	1	1
103	29	62.07	18	16/18 (89%)	1	0.78	0.82	1	0.78
105	29	96.55	28	28/28 (100%)	1	1	1	1	1
106	29	93.1	27	27/27 (100%)	1	1	1	1	1
107	29	100	29	29/29 (100%)	1	1	1	1	1
109	29	93.1	27	27/27 (100%)	1	1	1	1	1
111	29	62.07	18	18/18 (100%)	1	1	1	1	1
111a	29	93.1	27	27/27 (100%)	1	1	1	1	1
112	29	93.1	27	27/27 (100%)	1	1	1	1	1
113	29	82.76	24	24/24 (100%)	1	1	1	1	1
114	29	93.1	27	27/27 (100%)	1	1	1	1	1
115	29	96.55	28	28/28 (100%)	1	1	1	1	1
116	29	93.1	27	27/27 (100%)	1	1	1	1	1
117	29	96.55	28	28/28 (100%)	1	1	1	1	1
119	29	96.55	28	28/28 (100%)	1	1	1	1	1
120	29	96.55	28	27/28 (96%)	1	0.93	0.93	1	0.93
122	29	96.55	28	28/28 (100%)	1	1	1	1	1
123	29	100	29	29/29 (100%)	1	1	1	1	1
124	29	100	29	29/29 (100%)	1	1	1	1	1
125	29	100	29	29/29 (100%)	1	1	1	1	1
126	29	93.1	27	27/27 (100%)	1	1	1	1	1
127	29	100	29	28/29 (97%)	1	0.93	0.93	1	0.93
128	29	100	29	29/29 (100%)	1	1	1	1	1
129	29	100	29	29/29 (100%)	1	1	1	1	1
132	29	96.55	28	28/28 (100%)	1	1	1	1	1
133	29	100	29	29/29 (100%)	1	1	1	1	1
134	29	93.1	27	27/27 (100%)	1	1	1	1	1
135	29	75.86	22	21/22 (95%)	1	0.91	0.92	1	0.91
136	29	68.97	20	20/20 (100%)	1	1	1	1	1
138	29	72.41	21	21/21 (100%)	1	1	1	1	1
139	29	100	29	29/29 (100%)	1	1	1	1	1
141	29	93.1	27	25/27 (93%)	1	0.86	0.87	1	0.85
143	29	96.55	28	28/28 (100%)	1	1	1	1	1
145	29	68.97	20	20/20 (100%)	1	1	1	1	1
146	29	96.55	28	28/28 (100%)	1	1	1	1	1
147	29	96.55	28	28/28 (100%)	1	1	1	1	1
149	29	96.55	28	28/28 (100%)	1	1	1	1	1
150	29	96.55	28	28/28 (100%)	1	1	1	1	1
151	29	96.55	28	28/28 (100%)	1	1	1	1	1
152	29	96.55	28	28/28 (100%)	1	1	1	1	1
153	29	72.41	21	21/21 (100%)	1	1	1	1	1
155	29	89.66	26	25/26 (96%)	1	0.93	0.92	1	0.92
157	29	79.31	23	21/23 (91%)	1	0.83	0.85	1	0.83
159	29	96.55	28	22/28 (79%)	0.57	1	1	0.7	0.57
160	29	68.97	20	20/20 (100%)	1	1	1	1	1
161	29	96.55	28	28/28 (100%)	1	1	1	1	1
164	29	93.1	27	25/27 (93%)	1	0.85	0.88	1	0.85
165	29	89.66	26	24/26 (92%)	1	0.86	0.86	1	0.85
167	29	96.55	28	28/28 (100%)	1	1	1	1	1
168	29	100	29	28/29 (97%)	1	0.93	0.93	1	0.93
170	29	100	29	29/29 (100%)	1	1	1	1	1
173	29	96.55	28	28/28 (100%)	1	1	1	1	1
175	29	100	29	27/29 (93%)	1	0.87	0.88	1	0.86
177	29	89.66	26	26/26 (100%)	1	1	1	1	1
178	29	79.31	23	23/23 (100%)	1	1	1	1	1
183	29	89.66	26	26/26 (100%)	1	1	1	1	1
184	29	79.31	23	23/23 (100%)	1	1	1	1	1
184a	29	89.66	26	26/26 (100%)	1	1	1	1	1
186	29	89.66	26	26/26 (100%)	1	1	1	1	1
187	29	96.55	28	28/28 (100%)	1	1	1	1	1
189	29	82.76	24	24/24 (100%)	1	1	1	1	1
190	29	86.21	25	25/25 (100%)	1	1	1	1	1
191	29	82.76	24	23/24 (96%)	1	0.92	0.92	1	0.92
194	29	79.31	23	23/23 (100%)	1	1	1	1	1
196	29	93.1	27	27/27 (100%)	1	1	1	1	1
198	29	86.21	25	25/25 (100%)	1	1	1	1	1
199	29	82.76	24	23/24 (96%)	1	0.92	0.92	1	0.92
202	29	86.21	25	24/25 (96%)	1	0.92	0.92	1	0.92
207	29	96.55	28	25/28 (89%)	0.93	0.86	0.87	0.92	0.79
208	29	82.76	24	24/24 (100%)	1	1	1	1	1
209	29	96.55	28	28/28 (100%)	1	1	1	1	1
215	29	89.66	26	26/26 (100%)	1	1	1	1	1
216	29	82.76	24	24/24 (100%)	1	1	1	1	1
217	29	86.21	25	23/25 (92%)	1	0.85	0.86	1	0.84
221	29	96.55	28	28/28 (100%)	1	1	1	1	1

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	35	97.14	33	33/33 (100%)	1	1	1	1	1
102	35	94.29	32	32/32 (100%)	1	1	1	1	1
103	35	97.14	33	33/33 (100%)	1	1	1	1	1
105	35	91.43	31	31/31 (100%)	1	1	1	1	1
106	35	88.57	30	30/30 (100%)	1	1	1	1	1
107	35	94.29	32	32/32 (100%)	1	1	1	1	1
109	35	82.86	28	28/28 (100%)	1	1	1	1	1
111	35	94.29	32	32/32 (100%)	1	1	1	1	1
111a	35	97.14	33	33/33 (100%)	1	1	1	1	1
112	35	82.86	29	29/29 (100%)	1	1	1	1	1
113	35	94.29	32	32/32 (100%)	1	1	1	1	1
114	35	94.29	32	32/32 (100%)	1	1	1	1	1
115	35	85.71	29	29/29 (100%)	1	1	1	1	1
116	35	91.43	31	31/31 (100%)	1	1	1	1	1
117	35	91.43	31	31/31 (100%)	1	1	1	1	1
119	35	94.29	32	32/32 (100%)	1	1	1	1	1
120	35	85.71	30	30/30 (100%)	1	1	1	1	1
123	35	85.71	30	30/30 (100%)	1	1	1	1	1
124	35	97.14	33	33/33 (100%)	1	1	1	1	1
125	35	91.43	31	31/31 (100%)	1	1	1	1	1
126	35	97.14	33	33/33 (100%)	1	1	1	1	1
127	35	94.29	32	32/32 (100%)	1	1	1	1	1
129	35	85.71	29	29/29 (100%)	1	1	1	1	1
133	35	100	34	34/34 (100%)	1	1	1	1	1
135	35	97.14	33	33/33 (100%)	1	1	1	1	1
136	35	91.43	32	32/32 (100%)	1	1	1	1	1
138	35	65.71	23	23/23 (100%)	1	1	1	1	1
139	35	85.71	29	29/29 (100%)	1	1	1	1	1
145	35	88.57	31	31/31 (100%)	1	1	1	1	1
147	35	94.29	32	32/32 (100%)	1	1	1	1	1
149	35	82.86	28	28/28 (100%)	1	1	1	1	1
150	35	94.29	32	32/32 (100%)	1	1	1	1	1
151	35	80	27	27/27 (100%)	1	1	1	1	1
152	35	82.86	28	28/28 (100%)	1	1	1	1	1
153	35	91.43	31	31/31 (100%)	1	1	1	1	1
155	35	82.86	28	28/28 (100%)	1	1	1	1	1
157	35	97.14	33	33/33 (100%)	1	1	1	1	1
160	35	94.29	32	32/32 (100%)	1	1	1	1	1
161	35	71.43	25	25/25 (100%)	1	1	1	1	1
162	35	71.43	25	25/25 (100%)	1	1	1	1	1
164	35	77.14	26	26/26 (100%)	1	1	1	1	1
167	35	77.14	26	26/26 (100%)	1	1	1	1	1
168	35	74.29	25	25/25 (100%)	1	1	1	1	1
170	35	77.14	26	26/26 (100%)	1	1	1	1	1
175	35	94.29	32	32/32 (100%)	1	1	1	1	1
181	35	71.43	24	24/24 (100%)	1	1	1	1	1
186	35	91.43	31	31/31 (100%)	1	1	1	1	1
187	35	85.71	29	29/29 (100%)	1	1	1	1	1
189	35	97.14	33	33/33 (100%)	1	1	1	1	1
190	35	88.57	31	31/31 (100%)	1	1	1	1	1
191	35	91.43	31	31/31 (100%)	1	1	1	1	1
194	35	88.57	30	30/30 (100%)	1	1	1	1	1
198	35	94.29	32	32/32 (100%)	1	1	1	1	1
199	35	94.29	32	32/32 (100%)	1	1	1	1	1
202	35	85.71	30	30/30 (100%)	1	1	1	1	1
207	35	88.57	30	30/30 (100%)	1	1	1	1	1
215	35	91.43	31	31/31 (100%)	1	1	1	1	1
216	35	94.29	32	32/32 (100%)	1	1	1	1	1
217	35	91.43	32	32/32 (100%)	1	1	1	1	1
221	35	97.14	33	33/33 (100%)	1	1	1	1	1