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Assessors' report for cIQc Run 64: Breast Module (ER, PR and HER2)

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Assessment performed on October 14, 2016 at St. Paul's Hospital, Vancouver, British Columbia.

Overview

The TMA used in Run 64 was previously used for cIQc Run 11 (2009). The TMA was prepared by Dr. Nickolas Myles (Nikita Makretsov) and consists of unselected cases of invasive breast cancer drawn from St. John's, Newfoundland. The cIQc is delighted to report that no laboratories this challenge had staining that was considered below "adequate". Slides from Labs 112, 124, 147, and 189 were not returned in time for the assessment meeting.

ER

Core 3 was noted to have sampling variability with generally weak staining in few tumour cells, except when overstaining was noted (Figure 1). Since the majority of labs exhibited optimal staining, participant specific feedback is provided below only for labs for which a comment was made by the assessment team:

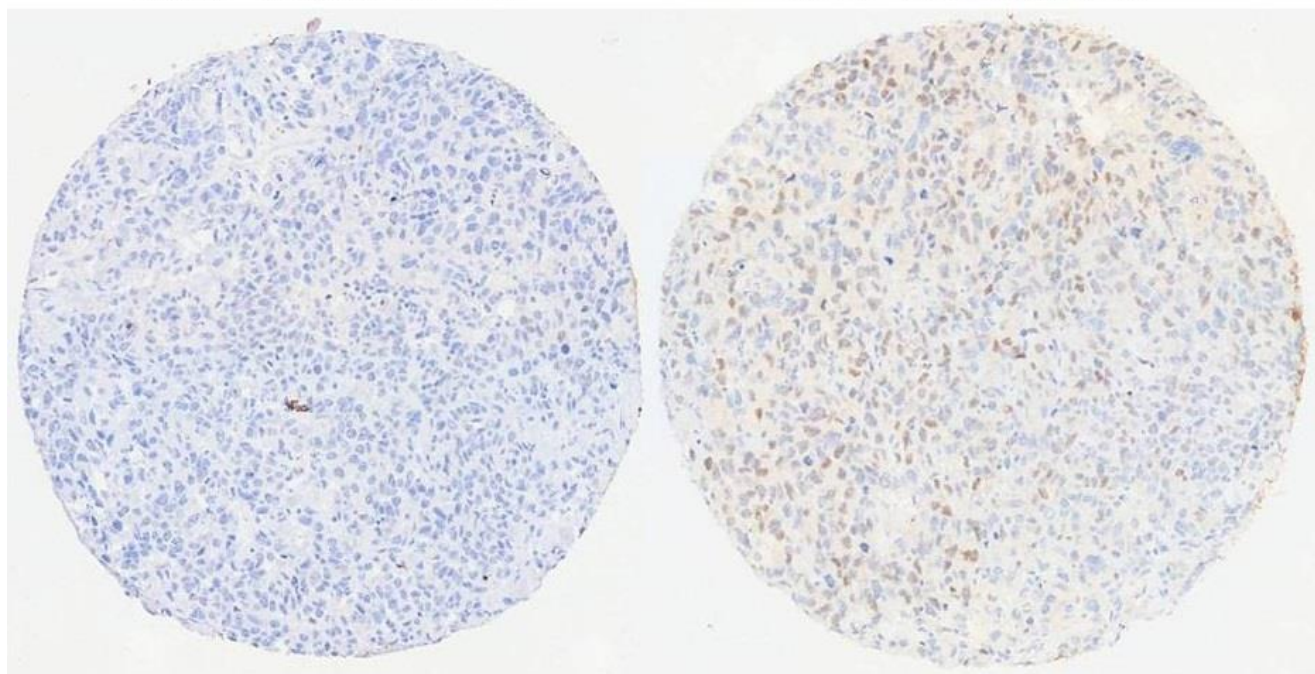
Lab ID	IHC Status*	Comments
101	Optimal	Nice intensity gradient observed
103	Optimal	Cytoplasmic background noted and faint counterstain
112	--	Slides not available
114	Optimal	Slightly overstained
115	Optimal	Nice intensity gradient observed; very crisp staining for easy interpretation
124	--	Slides not available
125	Optimal	Overstained
129	Optimal	Fibroblast staining noted
138	Optimal	Slightly over-digested (cores have jagged edges)
147	--	Slides not available
146	Adequate	Strong counterstain makes interpretation more challenging
151	Adequate	Overstained; lots of cytoplasmic background
183	Optimal	Strong counterstain that occasionally makes interpretation more challenging
189	--	Slides not available
221	Optimal	Strong counterstain that occasionally makes interpretation more challenging

*Based on cIQc assessor consensus

Figure 1. Representative images of the variability observed in Core 3.

Core 3 – Negative (Lab 115)

Core 3 – Positive (Lab 125)



PR

Core 5, 9, 24, and 40 were noted to have general sampling variability. Since the majority of labs exhibited optimal staining, participant specific feedback is provided below only for labs for which a comment was made by the assessment team:

Lab ID	IHC Status*	Comments
102	Adequate	Overstained
112	--	Slides not available
124	--	Slides not available
147	--	Slides not available
186	Optimal	Only lab with weak positive staining in Core 47 (note: only lab using Clone PR88)
189	--	Slides not available
199	--	Slides not available

*Based on cIQc assessor consensus

HER2

Similar to ER and PR, since the majority of labs exhibited optimal staining, participant specific feedback is provided below only for labs for which a comment was made by the assessment team:

Lab ID	IHC Status*	Comments
112	--	Slides not available
124	--	Slides not available
147	--	Slides not available
151	Adequate	Overall weaker staining compared to other labs, but interpretation unaffected.
189	--	Slides not available
190	Adequate	Counterstain faint
221	--	Unable to review slide due to a staining artifact seemingly caused by a bubble on the slide during staining.

*Based on cIQc assessor consensus

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. 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.

Figure S3. Garrattogram after cIQc assessment of HER2 IHC. (NOTE: orientation marker cores have been removed)

Lab/ Core	101	102	103	106	107	109	111	112	114	115	120	123	124	125	126	127	129	133	138	145	147	149	150	151	155	157	160	161	168	175	181	186	187	189	190	194	199	202	217	221	
1	N	1	2	2	1	1	2	2	N	2	2	2	N	1	1	2	1	2	2	1	2	2	1	1	1	2	1	1	2	2	2	2	2	2	2	N	2	1	2	2	2
2	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	1	N	U	N	N	N	1	N	1
3	N	N	N	N	N	N	1	1	N	1	1	N	N	1	1	1	N	1	1	1	1	N	N	1	1	1	1	1	1	1	1	N	1	N	1	U	1	N	N	N	1
4	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	
5	N	N	N	N	N	N	N	N	N	1	1	N	N	N	N	N	N	N	1	1	N	1	N	N	N	N	1	N	N	N	N	N	N	1	N	N	N	N	1	N	1
6	N	N	N	N	N	1	1	1	N	1	1	N	N	N	N	N	N	N	1	1	N	1	N	N	N	N	1	N	N	1	1	N	2	N	1	N	N	N	N	N	1
7	N	N	1	N	1	1	1	1	N	1	N	N	N	N	N	N	1	N	1	1	N	2	N	N	1	N	N	N	N	N	1	N	1	N	1	N	1	N	1	N	N
8	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	1	1	2	N	N	2	N	N	N	N	N	N	N	N	1	N	N	N	N	1	1	N	1
9	N	U	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
10	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
15	N	N	1	1	N	N	1	1	N	1	1	N	N	N	N	1	N	1	1	N	2	N	N	1	1	1	N	N	U	1	N	1	N	1	N	1	N	N	N	N	1
16	U	U	U	U	3	2	3	3	3	2	3	U	U	2	3	U	3	3	U	U	U	U	U	3	U	3	3	U	3	3	2	3	U	U	U	3	U	U	3	U	
17	N	1	1	N	1	N	1	1	N	1	1	N	N	N	1	1	N	1	1	1	2	N	N	N	N	1	N	1	1	N	N	1	N	1	N	1	N	1	N	1	
18	N	N	1	N	N	N	N	1	N	1	1	N	N	N	1	N	N	1	1	U	2	N	N	U	N	N	N	N	N	N	1	N	N	N	N	N	1	N	N	1	N
19	N	N	1	N	1	1	1	1	N	2	1	N	N	N	1	U	N	1	U	2	U	U	1	N	1	N	1	N	1	N	1	N	1	N	N	N	1	N	N	1	U
20	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	1	N	1	N	1	N	1	N	N	N	N	N	N	N	1	N	N	N	U	N	N	N	N
21	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	U	3	3	3	3	3	3	3	3	3	3	3	U	3	3	3	3
22	N	1	2	1	1	1	2	2	N	2	1	2	N	1	1	1	N	2	2	1	2	1	N	N	1	2	1	1	1	1	2	2	1	2	U	1	N	2	1	1	
23	N	U	1	U	N	N	N	U	N	U	N	U	N	N	N	N	N	N	1	N	U	N	N	2	N	N	N	N	U	N	N	U	U	N	N	N	N	N	N	1	
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28	N	N	2	N	1	N	1	1	N	2	1	1	N	N	1	N	1	2	N	1	N	N	1	N	1	N	1	N	U	1	2	N	1	N	2	N	1	1	2	N	1
29	N	U	U	N	N	U	N	U	N	1	N	U	U	U	U	U	U	1	U	U	U	U	U	U	N	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	
30	N	1	1	1	1	N	N	N	1	N	N	N	N	N	1	N	1	N	1	1	1	N	1	N	1	N	1	N	1	N	1	N	1	N	1	N	1	N	1	N	1
31	N	N	1	N	1	1	1	1	N	2	1	N	N	1	N	1	N	2	2	N	N	U	N	N	1	1	N	1	1	2	1	2	N	2	U	1	N	2	2	2	
32	N	U	N	U	U	U	N	N	N	N	N	N	N	N	N	N	N	N	U	N	U	U	N	N	N	N	N	U	N	U	U	U	U	U	U	U	U	U	U	U	
33	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	
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39	N	N	N	N	N	N	N	N	N	1	1	N	N	N	N	N	N	N	1	1	N	2	N	N	N	N	2	1	1	1	N	2	N	1	N	N	N	1	N	1	
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42	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
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44	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	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U
45	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
46	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	U	N	U	U	N	N	N	
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48	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
49	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
50	N	N	2	1	1	1	2	2	N	2	2	1	N	1	1	2	N	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	1	2	N	1	N	2	2	
51	N	U	N	U	N	N	N	U	U	U	U	N	U	N	U	N	N	N	N	N	N	N	N	N	U	N	N	U	U	N	N	N	N	N	N	N	U	N	N	N	N

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	sp1	1:50	Fisher	9101s1501m	32	optiview	n	y	dab
102	Dako PT module - High pH TRS	10/20/10	EP1	1:40	DAKO	10106793	30	DAKO Envision FLEX+	No	CUSO4	DAB+
103	CC1	64 MINS	SP1	Predilute	VENTANA	F07776	16 MINS	ULTRA VIEW	N	Y	DAB
106	CC1	64 min	SP1	Predilute	Ventana Roche	G2331	32 minutes	Optiview	no	no	DAB
107	ultra cc1	36	SP1	Predilute	Ventana	G00585	16	Ultraview DAB	N	Y	DAB
109	HIER high pH (CC1)	64 MIN	SP1	RTU	ROCHE	F10363	32 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	36 MIN	SP1	Predilute	VENTANA	602331	32 MIN	ULTRAVIEW	N	COPPER	DAB
112	Bond ER2 pH 9.0	20 minutes	SP1	1:150	ThermoFisher	QL2125711	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
114	CC1	32	SP1	1/50	Thermo Fisher	9101S1501J	16	Optiview	N	Copper	DAB
115	HIER	30 min	EP1	RTU	Dako	lot # 10112555	30 min	Envision Flex	n	n	DAB
120	Waterbath	20 minutes	EP1	RTU	Dako	10110172	20	Flex	No	No	DAB
122	HIER ER2 PH 9.0	20	SP1	RTU	VENTANA	E10753	15	BOND POLYMER REFINE	N	N	DAB
123	CC1	36 MIN	SP1	Predilute	VENTANA/ROCHE	F09522	32 MIN	ULTRAVIEW DAB	N	Y (COPPER)	DAB
124	CC1	60 min	SP1	1/100	Cell Marque	1422401B	32	Ultra view	N	N	DAB
125	Omnis EnV Flex, TRIS high Ph	30	SP1	Predilute	Roche	F09522	20	Omnis EnV Flex	Y	N	DAB
126	Steam Citrate, ph 6.0	45minutes	SP1	1:200	Thermo Scientific	9101S1604A	30 minutes	Dako Envision Plus	No	No	DAB Plus
127	Automated (CC1 on Benchmark Ultra	36	SP1	Predilute	VENTANA	F07779	32	ULTRAVIEW DAB DETECTION KIT	N	N	DAB
128	CC1	64 min	SP1	Predilute	Ventana	G02331	16 min	Ultraview	No	Yes	DAB
129	ER2 - high pH retrieval	20	SP1	1:100	Thermo Scientific	9101S1501J	15	Bond Refine Detection Kit	N	N	DAB
132	Flex TRS high	20 min	EP1	RTU	DAKO	10112555	20 min	Envision Flex	n	n	DAB
133	hier	36	SP1	Predilute	Roche	F05106	32	polymer	n	n	dab
138	HIER - EDTA	20	EP1	RTU	Dako	10112555	20	Polymer	N	N	DAB
141	CC1-HIER	30 min	SP1	RTU	Ventana/Roche	F00389	8 min	ULTRAVIEW	N	N	DAB
144	CC1	24 min	SP1	1:50	ThermoScientific	9101S1604A	16 min	Optiview	No	Yes, copper	DAB
145	CC1	32	SP1	1/100	CELLMARQUE	1422401B	16	XT OPTIVIEW ihc v4	n	n	DAB
146	FLEX TRS High	20	EP1	RTU	DAKO	10106139	20	EnVision FLEX	No	No	DAB
147	HIER, pH9	20	SP3	1:150	Thermo Scientific	9101S1501J	15	Polymer (Leica refine)	N	N	DAB
148	CC1	36	SP1	N/A	VENTANA	F10363	12 MIN	ULTRAVIEW	N	N	DAB
149	PT Link high pH	20 min at 97 C	EP1	RTU	Dako	10112555	20	EnVision Flex	Yes	No	DAB
150	cc1	30min	SP1	n/a	ventana	910351407E	16min	ultraview	n	y	DAB
151	BUFFER PH 9.0	20MIN	SP1	1:50	THERMO FISHER	9101S1501J	15 MIN	BOND REFINE	N	N	DAB
155	CC1	30	SP1	Predilute	Ventana	G02331	40	Ultraview	n	y	Dab
157	CC1	24 MIN.	SP-1	Predilute	VENTANA	G02331	24 MIN.	OPTIVIEW BENCHMARK XT	Y	Y	DAB
160	CC1	36 MIN	SP1	Predilute	VENTANA	F09522	8 MIN	ULTRA-VIEW	N	CUSO4	DAB
161	HIER-High EDTA TRIS tampon	20 Minutes	EP1	RTU	DAKO	10114730	20 Minutes	Envision Flex	No	No	DAB
168	Cell conditioning (CC)	60 min	SP1	RTU	Roche	F02583	12 min	Ultraview DAB	N	Y	DAB
175	HIER	36	SP1	Pre-dilute	Roche	G02331	32	Polymer	N	Y	DAB
178	HIER	32	SP1	None	Ventana	na	16	Ultraview	N	N	DAB
183	Ultra CC1	36	SP1	predilute	Ventana	F08061	32 min	Ultraview	n	n	DAB
186	HIER	20 MIN	SP1	1:50	THERMOSCIENTIFIC	9101S1501E	15 MIN	LEICA	N	N	DAB
187	CC1	16	SP1	None	Roche	G02331	8	Optiview	None	None	DAB
189	CC1	64	SP1	RTU	Ventana	unknown	16	UltraView	N	N	UltraView DAB
190	cc1	32	sp1	Predilute	ventana	F08061	32	iview	N	N	DAB
192	Ultra CC1	36 minutes	SP1	RTU	Ventana/Roche	F08061	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	SP1	Predilute	ROCHE/VENTANA	F09522	12	IVIEW KIT(AVIDIN-BIOTIN)	Y	Y	DAB
196	NONE	0	SP1	0	VENTANA	F08061	8	DAB	N	N	N
199	HIER BOND ER-1	20	6F11	RTU	LEICA	42449	15	BOND REFINE (POLYMER)	N	N	DAB
202	Leica ER2 citrate Buffer 9.5	20 min	6f11	1/300	vector	6027092	15 min	Refine Detection kit Leica	no	no	DAB
209	HIER	20mins at97C and then 20mins coling to 85C	EP1	Predilute	Dako	10114730	20mins	Envision Plus	N	N	DAB
217	HIER	64	SP1	RTU	Roche/Ventana	790-4325	20	Optiview	N	Y	DAB
221	pH6 Citrate Buffer	20	SP1	1:100	Cell Marque	1603507B	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	16	1:100	leica	6015355	32	optiview	n	y	dab
102	DAKO PT Module - High pH TRS	10/20/10	16	1:150	Novocastra	6027462	30	Dako Envision FLEX+	No	CUSO4	DAB+
103	CC1	64 MINS	100	Predilute	VENTANA	G00595	16 MINS	ULTRA VIEW	N	Y	DAB
106	CC1	64 min	PgR 1294	1:150	Dako	10109383	32 minutes	Optiview	no	no	DAB
107	ultra cc1	64	PgR 1294	1:50	Dako	10112568	23	Ultraview DAB	Y	Y	DAB
109	HIER high pH (CC1)	36 MIN	Pgr 1294	1/50	DAKO	10092055	16 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	48 MIN	16	1/80	LEICA	6041139	32 MIN	OPTIVIEW	N	COPPER	DAB
112	bond er2 Ph 9.0	12 minutes	16	RTU	Leica	45447	15 minutes @ RT	BOND POLYMER REFINE	NO	NO	DAB
114	CC1	32	16	1/25	Novocastra	6027462	16	Optiview	N	Y	DAB
115	HIER	30 min	PgR 1294	1/50	Dako	lot# 10104594	20 mins	Envision Flex	N	N	DAB
120	Waterbath	20	636	RTU	Dako	10096659	20	Flex	Yes	No	DAB
122	HIER ER2 PH 9.0	20	16	RTU	LEICA/NOVOCASTRA	41343	15	BOND POLYMER REFINE	N	N	DAB
123	CC1	64 MIN	16	1/25	LEICA	6041139	60 MIN	ULTRAVIEW DAB	N	Y (COPPER)	DAB
124	CC1	30 min	100	Predilute	Ventana	G01431	12 min	Ultra view	N	N	DAB
125	CC1	36	1.00E+02	Predilute	Roche	F07776	8	Ultraview	N	Y	DAB
126	Steam Citrate, ph 6.0	45minutes	636	1:500	Dako	10110361	30 minutes	Dako Envision Plus	no	no	DAB Plus
127	Automated (CC1 on Benchmark Ultra)	36	100	Predilute	VENTANA	F05104	8	ULTRAVIEW DAB DETECTION KIT	N	N	DAB
128	CC1	64 min	100	Predilute	Ventana	G01431	16 min	Ultraview	No	Yes	DAB
129	ER2 - high pH retrieval	20	16	1:400	Novocastra	6027295	15	Bond Refine Detection Kit	N	N	DAB
132	Flex TRS High	20 min	PR16	1:200	Vector	6024555	30 min	Envision Flex	N	N	DAB
133	hier	64	16	1/25	Leica	6031757	60	polymer	n	n	dab
134	CC1 - HIER	30 min	100	RTU	VENTANA/ROCHE	F00392	12 min	ULTRAVIEW	N	N	DAB
138	HIER - EDTA	20	636	RTU	Dako	10109504	20	Polymer	N	N	DAB
141	CC1 -HIER	30 min	100	RTU	Ventana/Roche	F00392	12min	ULTRAVIEW	N	N	DAB
145	CC1	32	16	1/100	NOVOCASTRA	6027462	24	XT OPTIVIEW ihc v4	n	n	DAB
146	Flex TRS High	20	636	RTU	Dako	10101557	30	EnVision FLEX	No	No	DAB
147	HIER, ph9	20	SP3	1:800	Novocastra	6027295	15	Polymer (Leica refine)	N	N	DAB
149	PT Link high pH	20 min at 97 C	PgR636	RTU	Dako	10107676	20	EnVision Flex	Yes	No	DAB
150	cc1	30min	100	n/a	ventana	G01431	20min	ultraview	n	y	DAB
151	BUFFER PH 6.0	20 MIN	1A6	1:200	NCL	6027295	15 MIN	BOND REFINE	N	N	DAB
155	CC1	30	100	Predilute	Ventana	G01431	32	Ultraview	n	y	dab
157	CC1	24 MIN.	IE2	Predilute	VENTANA	F10358	24 MIN.	OPTIVIEW BENCHMARK XT	Y	Y	DAB
160	CC1	36 MIN	100	Predilute	VENTANA	F09520	8 MIN	ULTRA-VIEW	N	CUSO4	DAB
161	HIER-High EDTA TRIS tampon	20 Minutes	PgR 636	RTU	DAKO	10109504	20 Minutes	Envision Flex	No	No	DAB
168	HIER	48 min	PGR636	RTU	Dako	10114064	20 min	Envision Flex+	N	Y	DAB
175	HIER	64	100	Pre-dilute	Roche	F10358	32	Polymer	N	Y	DAB
178	HIER	32	100	None	Ventana	NA	16	Ultraview	n	n	DAB
183	Ultra CC1	36 min	100	Predilute	Ventana	F10358	32 min	ultraview	n	n	DAB
186	HIER	20 MIN	PR88	1:100	BIOGENEX	MU3280415	15 MIN	LEICA	N	N	DAB
187	CC1	64	IE2	Predilute	Roche	F02563	12	Ultraview	N	N	DAB
189	CC1	64	100	RTU	Ventana	unknown	16	UltraView	N	N	UltraView DAB
190	cc1	32	16	1:50	Novocastra	6027462	32	iview	N	N	DAB
192	Ultra CC1	36 minutes	100	RTU	Ventana/Roche	F09470	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	100	Predilute	ROCHE/VENTANA	F09520	20	IVIEW KIT(AVIDIN-BIOTIN)	N	Y	DAB
196	NONE	0	100	0	VENTANA	F07130	8	DAB	N	N	N
199	HIER BOND ER-2	20	16	200	LEICA	6027462	15	BOND REFINE (POLYMER)	N	N	DAB
202	citrate pH9.5 Leica	30 min	16	RTU Leica	Leica	45139	15 min	Refine Detection kit Leica	no	no	DAB
209	HIER	20mins at 97C and then 20mins cooling down to 85C	PgR636	Predilute	Dako	10114064	20mins	Envision Plus	Y	N	DAB
217	HIER	64	100	RTU	Roche/Ventana	790-4296	16	Optiview	N	Y	DAB
221	pH6 Citrate Buffer	20	1A6	1:200	Leica	6041139	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	Time for Ab Incubation (min)	Ab Lot No.	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	cc1	32	sp3	1:200	Fisher	32	9103s1306a	Optiview	no	y	dab
102	DAKO PT module - High pH TRS	10/20/10	SP3	1:125	LABVISION - THERMO	30	9103S1509M	DAKO ENVISION FLEX+	NO	CUSO4	DAB+
103	CC1	36 MINS	4B5	PRE	VENTANA	16 MINS	G03109	ULTRA VIEW	N	Y	DAB
106	CC1	36 min	4B5	predilute	Roche/ Ventana	16 min	G00583	ultraview	no	no	DAB
107	ultra cc1	36	4B5	Pre-diluted	Ventana	8	G02207	Ultraview DAB	N	Y	DAB
109	HIER high pH (CC1)	36 MIN	4B5	RTU	ROCHE	16 MIN	G00135	ULTRAVIEW	N	Y	DAB
111	CC1	36 MIN	4B5	Predilute	Ventana	32 MIN	G02207	ULTRAVIEW	N	COPPER	DAB
112	BOND ER2 pH 9.0	20 minutes	4B5	1:3 ratio of the RTU	Ventana	15 minutrs @ RT	G00135	Bond Polymer Refine	No	No	DAB
114	CC1	32	SP3	1/200	Thermo Fisher	32	9103S1509D	Optiview	N	Y	DAB
115	HIER	40 min	Hercept test	RTU	Dako	30 min	lot # 20035244	Herceptest	N	N	DAB
120	Waterbath	40	Herceptest	RTU	Dako	30	20031056	Flex	No	No	DAB
123	CC1	36 MIN	4B5	PREDILUTE	VENTANA/ROCHE	24 MIN	G04794	ULTRAVIEW DAB	N	Y (COPPER)	DAB
124	CC1	8 min	4B5	PrÃ©-diluÃ©	Ventana	20	G02207	Ultra view	N	N	DAB
125	CC1 mild	36	4B5	PREDILUE	Roche	16	G02342	Ultraview	N	Y	DAB
126	Steam, 30 minutes Tris, ph 10	55 minutes	SP3	1:200	Thermo Scientific	30 minutes	9103S1606E	Envision Plus	No	No	DAB Plus
127	Automated (CC1 on Benchmark Ultra)	36	4B5	PREDILUTE	VENTANA	24	G03109	ULTRAVIEW DAB DETECTION KIT	N	N	DAB
129	ER2 - high pH retrieval	20	SP3	1:100	Thermo Scientific	15	RA2136461	Bond Refine Detection Kit	N	N	DAB
133	hier	36	4B5	predilute	Roche	24	G00558	polymer	n	n	dab
138	HIER - Citrate	40	HercepTest	RTU	Dako	30	20032044	Polymer	N	N	DAB
145	CC1	32	SP3	1/1000	CELLMARQUE	40	1332302A	XT OPTIVIEW ihc v4	n	n	DAB
147	HIER, ph9	20	SP3	1:50	Thermo Scientific	15	RE2204071	Polymer (Leica refine)	N	N	DAB
149	PT Link low pH	20 min at 97 C	Poly R	1:500	Dako	20	20027850	EnVision Flex	Yes	No	DAB
150	cc1	30min	SP-3	1/100	NeoMarkers	20min	F06378	ultraview	n	y	DAB
151	BUFFER PH 6.0	20 MIN	SP3	1:100	THERMO	15 MIN	RA2131961	BOND REFINE	N	N	DAB
155	CC1	30	4B5	Predilute	Ventana	32	G00135	Ultraview	n	y	DAB
157	CC1	24 MIN.	4B-5	PRE DILUTED	VENTANA	24 MIN.	G01245	OPTIVIW BENCHMARK XT	Y	Y	DAB
160	CC1	36 MIN	4B5	PRE-DILUTED	VENTANA	16 MIN	G03109	ULTRA-VIEW	N	CUSO4	DAB
161	Herceptest epitope	40 Minutes	Rabbit anti-human HER2 protein	RTU	DAKO	30 Minutes	20032044	Herceptest visualization reagent	No	No	Herceptest DAB chromogene
168	Cell Conditioning	30 min	4B5 Pathway	RTU	Roche	16 min	G03109	Ultraview DAB	N	Y	DAB
175	HIER	32	4B5	Pre-dilute	Roche	16	G02342	Polymer	N	Y	DAB
181	CC1 on board	30 minutes	4B5	pre-diluted	Ventana/Fisher	16 minutes	F09740	Ventana Ultraview DAB	no	yes	DAB
186	HIER	20 MIN.	POLYCLONAL	1:400	DAKO	15 MIN	20023582	LEICA	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	24	G04794	Optiview	N	N	DAB
189	CC1	32	4B5	RTU	Ventana	16	unknown	UltraView	N	N	UltraView DAB
190	cc1	32	sp3	1:50	Thermofisher	40	9103s1507	IView	Y	N	DAB
194	CC1	30	4B5	PREDILUTE	ROCHE/VENTANA	12	G03798	IVIEW KIT(AVIDIN-BIOTIN)	N	Y	DAB
199	HIER BOND ER-2	20	SP3	300	CELL MARQUE	15	1602506A	BOND REFINE (POLYMER)	N	N	DAB
202	Herceptest test Epitope Retrieval ph 6.0	40 min	Her2	RTU DAKO Herceptest	DAKO	30 min	20027876	Herceptest kit DAKO	no	no	DAB
217	HIER	32	4B5	RTU	Roche/Ventana	20	740-4493	Optiview	N	Y	DAB
221	Dako Visualization Solution	40	Dako Hercep Test	NEAT	Dako	30	20035214	Dako Kit	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	43	93.02	39	39/39 (100%)	1	1	1	1	1
102	43	83.72	36	36/36 (100%)	1	1	1	1	1
103	43	90.7	39	39/39 (100%)	1	1	1	1	1
106	43	90.7	38	38/38 (100%)	1	1	1	1	1
107	43	93.02	38	38/38 (100%)	1	1	1	1	1
109	43	90.7	38	38/38 (100%)	1	1	1	1	1
111	43	93.02	38	38/38 (100%)	1	1	1	1	1
112	43	88.37	37	37/37 (100%)	1	1	1	1	1
114	43	93.02	38	38/38 (100%)	1	1	1	1	1
115	43	86.05	37	37/37 (100%)	1	1	1	1	1
120	43	88.37	37	37/37 (100%)	1	1	1	1	1
122	43	93.02	38	38/38 (100%)	1	1	1	1	1
123	43	86.05	36	36/36 (100%)	1	1	1	1	1
124	43	88.37	38	38/38 (100%)	1	1	1	1	1
125	43	90.7	38	37/38 (97%)	1	0.83	0.97	1	0.89
126	43	90.7	38	38/38 (100%)	1	1	1	1	1
127	43	90.7	38	38/38 (100%)	1	1	1	1	1
128	43	86.05	37	37/37 (100%)	1	1	1	1	1
129	43	93.02	38	37/38 (97%)	1	0.83	0.97	1	0.89
132	43	83.72	36	36/36 (100%)	1	1	1	1	1
133	43	93.02	39	39/39 (100%)	1	1	1	1	1
134	43	88.37	38	38/38 (100%)	1	1	1	1	1
138	43	81.4	35	35/35 (100%)	1	1	1	1	1
141	43	88.37	38	38/38 (100%)	1	1	1	1	1
144	43	88.37	38	38/38 (100%)	1	1	1	1	1
145	43	88.37	38	37/38 (97%)	1	0.86	0.97	1	0.91
146	43	81.4	35	35/35 (100%)	1	1	1	1	1
147	43	95.35	39	37/39 (95%)	1	0.71	0.94	1	0.8
148	43	86.05	37	37/37 (100%)	1	1	1	1	1
149	43	81.4	35	35/35 (100%)	1	1	1	1	1
150	43	86.05	37	37/37 (100%)	1	1	1	1	1
151	43	90.7	38	37/38 (97%)	1	0.83	0.97	1	0.89
155	43	90.7	39	39/39 (100%)	1	1	1	1	1
157	43	95.35	39	39/39 (100%)	1	1	1	1	1
160	43	93.02	39	39/39 (100%)	1	1	1	1	1
161	43	88.37	38	38/38 (100%)	1	1	1	1	1
168	43	95.35	39	39/39 (100%)	1	1	1	1	1
175	43	88.37	37	37/37 (100%)	1	1	1	1	1
178	43	86.05	37	37/37 (100%)	1	1	1	1	1
183	43	90.7	38	38/38 (100%)	1	1	1	1	1
186	43	90.7	39	38/39 (97%)	1	0.86	0.97	1	0.91
187	43	83.72	36	36/36 (100%)	1	1	1	1	1
189	43	90.7	39	38/39 (97%)	1	0.86	0.97	1	0.91
190	43	86.05	37	37/37 (100%)	1	1	1	1	1
192	43	90.7	39	39/39 (100%)	1	1	1	1	1
194	43	83.72	36	36/36 (100%)	1	1	1	1	1
196	43	90.7	38	38/38 (100%)	1	1	1	1	1
199	43	86.05	37	37/37 (100%)	1	1	1	1	1
202	43	90.7	39	39/39 (100%)	1	1	1	1	1
209	43	81.4	35	35/35 (100%)	1	1	1	1	1
217	43	93.02	39	38/39 (97%)	1	0.86	0.97	1	0.91
221	43	90.7	39	39/39 (100%)	1	1	1	1	1

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	43	90.7	38	36/38 (95%)	0.96	0.91	0.96	0.91	0.87
102	43	83.72	36	35/36 (97%)	1	0.89	0.96	1	0.92
103	43	88.37	38	37/38 (97%)	1	0.91	0.96	1	0.93
106	43	88.37	36	35/36 (97%)	1	0.89	0.96	1	0.92
107	43	86.05	36	35/36 (97%)	1	0.89	0.96	1	0.92
109	43	90.7	38	38/38 (100%)	1	1	1	1	1
111	43	95.35	39	39/39 (100%)	1	1	1	1	1
112	43	86.05	36	34/36 (94%)	0.92	1	1	0.83	0.87
114	43	93.02	38	37/38 (97%)	0.96	1	1	0.91	0.93
115	43	88.37	38	38/38 (100%)	1	1	1	1	1
120	43	90.7	38	35/38 (92%)	0.96	0.8	0.93	0.89	0.79
122	43	86.05	36	35/36 (97%)	1	0.89	0.96	1	0.92
123	43	79.07	34	33/34 (97%)	1	0.88	0.96	1	0.91
124	43	86.05	37	36/37 (97%)	1	0.9	0.96	1	0.93
125	43	86.05	37	36/37 (97%)	1	0.9	0.96	1	0.93
126	43	88.37	37	35/37 (95%)	0.92	1	1	0.85	0.88
127	43	88.37	37	37/37 (100%)	1	1	1	1	1
128	43	88.37	37	37/37 (100%)	1	1	1	1	1
129	43	86.05	37	36/37 (97%)	1	0.91	0.96	1	0.93
132	43	90.7	39	36/39 (92%)	1	0.73	0.9	1	0.79
133	43	83.72	36	32/36 (89%)	0.93	0.78	0.93	0.78	0.7
134	43	83.72	36	34/36 (94%)	0.96	0.9	0.96	0.9	0.86
138	43	88.37	37	35/37 (95%)	0.93	1	1	0.83	0.87
141	43	93.02	39	38/39 (97%)	0.96	1	1	0.92	0.94
145	43	86.05	37	37/37 (100%)	1	1	1	1	1
146	43	90.7	39	39/39 (100%)	1	1	1	1	1
147	43	86.05	37	34/37 (92%)	1	0.7	0.9	1	0.77
149	43	88.37	38	37/38 (97%)	1	0.9	0.97	1	0.93
150	43	81.4	35	34/35 (97%)	0.96	1	1	0.91	0.93
151	43	93.02	39	38/39 (97%)	1	0.91	0.97	1	0.93
155	43	83.72	36	36/36 (100%)	1	1	1	1	1
157	43	93.02	38	36/38 (95%)	0.96	0.91	0.96	0.91	0.87
160	43	90.7	38	37/38 (97%)	0.96	1	1	0.92	0.94
161	43	88.37	38	38/38 (100%)	1	1	1	1	1
168	43	90.7	37	37/37 (100%)	1	1	1	1	1
175	43	90.7	38	37/38 (97%)	0.96	1	1	0.91	0.93
178	43	86.05	36	35/36 (97%)	0.96	1	1	0.92	0.94
183	43	93.02	38	38/38 (100%)	1	1	1	1	1
186	43	93.02	39	38/39 (97%)	1	0.91	0.97	1	0.93
187	43	79.07	33	32/33 (97%)	0.96	1	1	0.89	0.92
189	43	83.72	36	34/36 (94%)	0.96	0.9	0.96	0.9	0.86
190	43	81.4	35	33/35 (94%)	0.92	1	1	0.83	0.87
192	43	83.72	36	35/36 (97%)	0.96	1	1	0.91	0.93
194	43	86.05	37	36/37 (97%)	1	0.89	0.97	1	0.92
196	43	90.7	38	38/38 (100%)	1	1	1	1	1
199	43	90.7	39	39/39 (100%)	1	1	1	1	1
202	43	88.37	38	38/38 (100%)	1	1	1	1	1
209	43	81.4	35	34/35 (97%)	0.96	1	1	0.9	0.93
217	43	93.02	39	39/39 (100%)	1	1	1	1	1
221	43	90.7	39	39/39 (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	43	93.02	40	40/40 (100%)	1	1	1	1	1
102	43	81.4	35	35/35 (100%)	1	1	1	1	1
103	43	90.7	39	39/39 (100%)	1	1	1	1	1
106	43	83.72	36	36/36 (100%)	1	1	1	1	1
107	43	93.02	40	40/40 (100%)	1	1	1	1	1
109	43	90.7	39	39/39 (100%)	1	1	1	1	1
111	43	95.35	41	41/41 (100%)	1	1	1	1	1
112	43	88.37	38	38/38 (100%)	1	1	1	1	1
114	43	93.02	40	40/40 (100%)	1	1	1	1	1
115	43	90.7	39	39/39 (100%)	1	1	1	1	1
120	43	93.02	40	40/40 (100%)	1	1	1	1	1
123	43	86.05	37	37/37 (100%)	1	1	1	1	1
124	43	88.37	38	38/38 (100%)	1	1	1	1	1
125	43	93.02	40	40/40 (100%)	1	1	1	1	1
126	43	93.02	40	40/40 (100%)	1	1	1	1	1
127	43	88.37	38	38/38 (100%)	1	1	1	1	1
129	43	95.35	41	41/41 (100%)	1	1	1	1	1
133	43	93.02	40	40/40 (100%)	1	1	1	1	1
138	43	86.05	37	37/37 (100%)	1	1	1	1	1
145	43	86.05	37	37/37 (100%)	1	1	1	1	1
147	43	88.37	38	38/38 (100%)	1	1	1	1	1
149	43	83.72	36	36/36 (100%)	1	1	1	1	1
150	43	83.72	36	36/36 (100%)	1	1	1	1	1
151	43	83.72	36	36/36 (100%)	1	1	1	1	1
155	43	93.02	40	40/40 (100%)	1	1	1	1	1
157	43	93.02	40	40/40 (100%)	1	1	1	1	1
160	43	93.02	40	40/40 (100%)	1	1	1	1	1
161	43	88.37	38	38/38 (100%)	1	1	1	1	1
168	43	86.05	37	37/37 (100%)	1	1	1	1	1
175	43	88.37	38	38/38 (100%)	1	1	1	1	1
181	43	93.02	40	40/40 (100%)	1	1	1	1	1
186	43	90.7	39	39/39 (100%)	1	1	1	1	1
187	43	83.72	36	36/36 (100%)	1	1	1	1	1
189	43	83.72	36	36/36 (100%)	1	1	1	1	1
190	43	81.4	35	35/35 (100%)	1	1	1	1	1
194	43	88.37	38	38/38 (100%)	1	1	1	1	1
199	43	86.05	37	37/37 (100%)	1	1	1	1	1
202	43	83.72	36	36/36 (100%)	1	1	1	1	1
217	43	95.35	41	41/41 (100%)	1	1	1	1	1
221	43	81.4	35	35/35 (100%)	1	1	1	1	1