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cIQc

canadian Immunohistochemistry Quality control

Assessors' report for cIQc Run 93: ER, PR and HER2

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Assessment performed on February 28, 2019-March 3, 2019 at Vancouver General Hospital, Vancouver, BC.

OVERVIEW

The Run 93 Breast Module (ER, PR, and HER2) consisted of three different breast carcinoma tissue microarrays (TMAs), with participants instructed to stain both an 80-core and 38-core TMA for ER (Run 93 Part 1) followed by a 48-core TMA for PR and HER2 (Run 93 Part 2). Unfortunately, delivery of unstained slides to several participating laboratories was significantly delayed due to a Canada Post postal strike and the deadline for submission of results was extended to January 31, 2019. Based on the self-assessment results of ER, PR and HER2 staining it was anticipated that overall results would be good and this expectation was, in fact, exceeded!

ER

ER TMA1 consisted of a single core tissue microarray containing 38 consecutive breast carcinoma cases. Core 31 had few tumours cells for most participants, leading to some heterogeneity of staining results across laboratories. ER TMA2 consisted of a duplicate-core tissue microarray containing 20 triple negative and 20 non-triple negative cases (according to pathology charts). Specifically, a trend of weak ER positive cases (i.e. Allred scores 3-5) was expected on this particular array, most notably in cores 39/40. Core 72 had few tumours cells for most participants, leading to heterogeneity of staining results across laboratories. All participating laboratories received an "Optimal" IHC Status, and no specific comments were noted by the assessment team for individual laboratories. Congratulations!

Garrattogram after cIQc assessment of ER TMA1:

Table with 38 rows (Lab/Core) and 38 columns (Lab/Core). Cells contain 'P' (Pass) or 'N' (Fail) in various colors (green, yellow, red) representing assessment results for ER TMA1.

PR

As there is no accepted “gold standard”, the consensus of results from participants remains the cIQc approach to determine reference result. Notably, Core 30 had variable PR positivity that was observed for laboratories using the 1E2 antibody clone. Sampling variability and/or weak positivity was evident for a small number of cores. In any weakly staining core the assessment team tried to be consistent, but acknowledges the interpretive challenges associated with weak, focal positivity. All labs exhibited either optimal or adequate staining. Participant-specific feedback is summarized below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Optimal	
103	Optimal	
106	Optimal	
107	Adequate	Slight blush
109	Optimal	
111	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	
120	Optimal	
123	Optimal	
125	Optimal	
127	Optimal	
128	Optimal	
129	Optimal	
132	Optimal	Nice staining
133	Optimal	
134	Optimal	
138	Optimal	
141	Adequate	Slight blush

Lab ID	IHC Status*	Comment
147	Optimal	Nice staining
149	Adequate	Slightly weak
151	Optimal	
175	Optimal	
178	Optimal	
183	Optimal	
186	Optimal	
187	Adequate	Slight weak with blush in some cores
189	Adequate	Slight blush
190	Adequate	Slightly weak
192	Optimal	
194	Optimal	
196	Optimal	
198	Optimal	
202	Optimal	
207	Optimal	
220	Optimal	
221	Optimal	
230	Optimal	
233	Optimal	

*Based on cIQc assessor consensus

HER2

No false positive or false negative results were observed on any slides that were returned in time for review at the assessment meeting. Participant-specific feedback is summarized below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
111	Optimal	
112	Optimal	
113	Optimal	Slightly weak
114	Optimal	
120	Optimal	
123	Optimal	
125	Optimal	
127	Optimal	
129	Optimal	
133	Optimal	
138	Optimal	
147	Optimal	

Lab ID	IHC Status*	Comment
149	Optimal	Nice staining
151	Optimal	
175	Optimal	
181	Optimal	
186	Optimal	
187	Optimal	
189	Optimal	
190	Optimal	
194	Optimal	
198	Optimal	
202	Optimal	
207	Optimal	
217	Optimal	
220	Optimal	
221	Adequate	Strong counterstain makes readout more challenging
230	Optimal	
233	Optimal	

*Based on cIQc assessor consensus

Supplementary Tables S1-S3 summarizing staining protocols and Supplementary Tables S4-S6 summarizing descriptive statistics can be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 7 provides the definitions of cIQc IHC Statuses assigned to each participant. 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	EnV FlexTRS, High PH	30 min	SP1	1:50	THERMO SCIENTIFIC	9101S1803C	30 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	ER	1:50	DAKO	10130521	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64	SP1	PRE	VENTANA	E12794	16	ULTRA VIEW	N	COPPER	DAB
106	CC1 on stainer	64	SP1	pre dilute	Roche	E10787	32	Optiview	Yes	yes	DAB
107	ultra cc1	36	SP1	Pre-diluted	Ventana	E12794	16	Ultraview DAB	N	Y	DAB
109	HIER high Ph	64 min	SP1	RTU	VENTANA/ROCHE	E10787	32 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	36	SP1	PREDILUTE	VENTANA	E14810	32	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	SP1	1:200	THERMOFISHER	9101S1711E	15 minutes @ RT	BOND Polymer Refine	no	no	DAB
113	CC1	32MIN	SP1	1/70	THERMO SCIENTIFIC	9101S1803C	32MIN	OPTIVIEW	Y	N	DAB
114	Envision Flex TRS, High pH	30	SP1	1:50	Thermo Fisher	9101S1711E	30	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	waterbath (TRS High)	20	EP1	RTU	Dako	10136915	20	Dako Envision Flex, flex20	N	N	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN.	SP1	PREDILUTE	VENTANA	Y24472	32 MIN.	ULTRAVIEW DAB	N	N	DAB
128	Ventana Ultra CC1	64 min	SP1	Ready-To-Use	Ventana/Roche	E10787	16 min	UltraView Universal Dab Detection Kit	N	N	CuSO4
129	ER 2- high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1803C	15	Bond Refine Detection Kit	N	N	DAB
132	Envision Flex, High pH	20	ER-EP1	RTU	Agilent	10132259	20	Envision Flex	N	N	DAB
133	Indirect biotin-free	36 min	SP1	pre-dilute	Ventana/Roche	E12794	32 min	Ultraview Lot E12794	N	Y	DAB
134	HIER - CC1	30 min	SP1	RTU	VENTANA/ROCHE	E03298	8 min	ULTRAVIEW	N	N	DAB
138	EDTA HIER	30	EP1	RTU	Dako	10138256	20	Polymer/Dako	N	N	DAB
141	HIER-CC1	30 min	SP1	RTU	VENTANA/ROCHE	E03298	8 min	ULTRAVIEW	N	N	DAB
144	CC1	24 min	SP1	1:50	Thermo Scientific	9101S711E	16 min	Optiview	n	y copper	Dab
147	HIER PH9	20	SP1	1:150	Thermo-Fisher	9101S11704e	15	Refine Leica Polymer	N	N	DAB
148	CC1	36 min	SP1	RTU	Ventana	E10787	12 min	Ultraview	NO	NO	DAB
149	high pH OMNIS	20 min 97 C	EP1	RTU	Dako	20058856	20	EnVision Flex	No	No	DAB
151	buffer ph 9.0	20	SP1	1:50	THERMO SCIENTIFIC	9101S1711E	15	BOND REFINE	N	N	DAB
175	HIER	36 mins	SP1	Predilute	Roche	E07836	32 mins	Ultra-DAB	N	4 (copper)	DAB
180	CC1	32	SP1	RTU	VENTANA	E10787	16	OPTIVIEW	N	Y	DAB
183	ULTRA CC1	36	SP1	RTU	VENTANA	E03296	32	ULTRAVIEW	N	N	DAB
186	HIER	20	SP1	1:50	Thermo Scientific	9101S1711E	15	Leica Bond Polymer Refine Kit	N	N	DAB
187	CC1	16	SP1	Predilute	Roche	E14810	8	Optiview	N	N	DAB
189	CC1	64	SP1	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32 (mild)	SP1 rabbit	Pre-dilute	Ventana	E10787	32	Ventana iView	N	N	DAB
192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	E10787	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	(HIER) CC1	64	SP1	RTU	ROCHE	E12794	16	ULTRAVIEW	N	Y	DAB
198	High pH HIER	30 min	EP1	Prediluted	Dako/Agilent	10136915	30 min	Envision Flex/HRP	N	N	DAB
202	er1	15	ER6F11	1/100	leica	6055347	15	Refine Detection system	no	no	DAB
207	CC1-on line	36 minutes	SP1	Prediluted	Ventana	E12794	16 minutes	Ultra view	N	Y	DAB
209	HIER High PH	20	EP1	none	Dako	10139304	30	Envision Polymer	No	No	DAB
217	hier	64	sp1	rtu	ventana	e07983	20	optiview	n	y	dab
220	CC1	36 min	SP1	Pre-Dilute	Roche	E14810	36 min	UltraView	N	N	DAB
221	pH 6 Citrate	20	SP1	1:50	Cell Marque	23235	30	Biocare Mach IV	N	N	DAB
230	HIER	64	SP1	PREDILUTE	Ventana	E14810	32	OPTIVIEW	N	N	DAB
233	CC1	36mins	SP1	NA	Roche	E07836	16mins	ULTRAVIEW	N	Y	DAB

Table S2. Reported PR staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, High PH	30 min	PgR 1294	RTU	Dako	GA090	25 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	16	1:200	NOVOCASTRA	6052038	30" RT	DAKO ENVISION FLEX	NO	CUSO4	DAB+
103	CC1	64	1E2	pre	Ventana	E12265	16	ULTRA VIEW	N	Y COPPER	DAB
106	CC1 on stainer	64	PgR 1294	1/220	Dako	10125926	32	Optiview	Yes	yes	DAB
107	ultra cc1	64	PgR1294	1:50	Dako	10136634	32	Ultraview DAB	Y	Y	DAB
109	HIER high pH CC1	36 MIN	1294	1/50	DAKO	10132621	16 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	32	16	1/80	LEICA	6057451	32	OPTIVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	10 minutes	16	RTU	Leica	63037	15 minutes	BOND Polymer Refine	no	no	DAB
113	Dako low pH	20min	PgR636	1/250	Dako	10128775	15min	Flex+20	Y	N	DAB
114	Envision Flex TRS, High pH	30	1294	RTU	DAKO	10136626	25	DAKO OMNIS Envision Flex	N	N	Envision Flex DAB
120	HIER Waterbath	20	PgR 636	n/a	Dako	10134386	20	Envision Flex+	y	n	DAB
123	CC1 on Ultra	64	16	1/25	Leica	6057451	60	Roche ultraView DAB	N	N	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN.	1E2	PREDILUTE	VENTANA	Y12992	8 MIN.	ULTRAVIEW DAB	N	N	DAB
128	Ventana Ultra CC1	64 min	1E2	Ready-To-Use	Ventana/Roche	E17507	16 min	UltraView Universal Dab Detection Kit	N	N	CuSO4
129	ER 2- high pH retrieval	20	16	1:400	Novocastra	6027295	15	Bond Refine Detection Kit	N	N	DAB
133	HIER	64	16	1/25	Leica	6058804	60	Ultraview	n	n	dab
134	HIER - CC1	30 min	1E2	RTU	VENTANA/ROCHE	Y26140	12 min	ULTRAVIEW	N	N	DAB
138	EDTA HIER	30	PgR 636	RTU	Dako	10138258	20	Polymer/Dako	N	N	DAB
141	HIER-CC1	30 min	IE2	RTU	VENTANA/ROCHE	Y26140	12 min	ULTRAVIEW	N	N	DAB
147	HIER PH 9	20	16	800	NCL	6027295	15	REFINE LEICA	N	N	DAB
149	high pH OMNIS	20 min 97 C	PgR1294	RTU	Dako	20061928	10	EnVision Flex	No	No	DAB
151	HIER1/BUFFER 6.0	20	16	1:100	NCL	6053394	15	BOND REFINE	N	N	DAB
175	HIER	64 mins	1E2	predilute	Roche	E12263	32 mins	polymer	N	Y(copper)	dab
183	ULTRA CC1	36	IE2	RTU	VENTANA	E04965	32	ULTRAVIEW	N	N	DAB
186	HIER	20	PR88	1:50	Biogenex	MU3280416	15	Leica Bond Polymer Refine Kit	N	N	DAB
187	CC1	64	IE2	Predilute	Roche	E17507	12	Optiview	N	N	DAB
189	CC1	64	1E2	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
192	Ultra CC1	36 minutes	1E2	Ready to use	Ventana/Roche	E12263	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	HIER (CC1)	64	IE2	RTU	ROCHE	E12265	16	ULTRAVIEW	N	Y	DAB
196	NONE	NONE	1E2	NONE	VENTANA	E05750	8 MINUTES	DAB	NONE	NONE	none
198	High pH HIER	30 min	1294	1/100	Dako/Agilent	10125926	20 min	Envision Flex/HRP	N	N	DAB
202	er1	20	16	rtu	leica	62210	15	Refine Detection system	no	no	DAB
207	CC1- On line	36 minutes	1E2	Prediluted	Ventana	E18135	36 minutes	Ultraview	N	Y	DAB
217	hier	64	1E2	rtu	ventana	e14808	16	optiview	n	y	dab
220	CC1	8 min	1E2	Pre-Dilute	Roche	E12263	12 min	ultraView	N	N	DAB
221	pH 6 Citrate	20	1A6	1:200	Leica	23235	30	Biocare Mach IV	N	N	DAB
230	HIER	64	1E2	PREDILUTE	Ventana	E17507	16	ULTRAVIEW	N	N	DAB
233	CC1	48mins	16	1/100	ROCHE	6052038	32mins	OPTIVEIW	N	Y	DAB

Table S3. Reported HER2 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, High PH	30 min	4B5	1:8	ROCHE (VANTANA)	Y28979	15 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	SP3	1:80	CELL MARQUE	47142	30" RT	DAKO ENVISION FLEX	NO	CUSO4	DAB+
103	CC1	36	4B5	PRE	VENTANA	Y13939	16	ULTR VIEW	N	Y COPPER	DAB
106	CC1 on stainer	36	4B5	pre dilute	Roche	E17552	16	Ultraview	Yes	yes	DAB
107	ultra cc1	64	4B5	Pre-diluted	Ventana	E18754	24	Ultraview DAB	N	Y	DAB
109	HIER high pH CC1	36 MIN	4B5	RTU	VENTANA/ROCHE	E17552	16 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	36	4B5	PREDILUTE	VENTANA	E16719	32	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	4B5	1:4 ratio of RTU	Ventana/Roche	Y21121	15 miutes @ RT	BOND Polymer Refine	no	no	DAB
113	CC1	30min	SP3	1/50	CELL MARQUE	23770	16MIN	ULTRAVIEW	N	Y	DAB
114	Envision Flex TRS, High pH	30	4B5	1:8	Ventana/Roche	E11991	15	DAKO OMNIS Envision Flex	N	N	Envision Flex DAB
120	HIER Waterbath	40	Herceptest	RTU	Dako	20059542	30	HerceptTest	n	n	DAB
123	CC1 on Ultra	36	4B5	predilute	Roche	E16719	24	Roche ultraView DAB	N	N	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN.	4B5	PREDILUTE	VENTANA	E17552	24 MIN.	ULTRAVIEW DAB	N	N	DAB
129	ER 2- high pH retrieval	20	SP3	1:50	Thermo Scientific	T12636164	15	Bond Refine Detection Kit	N	N	DAB
133	HIER	36	4B5	predilute	Roche	E16719	24	Ultraview	n	n	Dab
138	HIER pH 6.0	40	Herceptest	RTU	Dako	20059542	30	Hercept Test	N	N	DAB
147	HIER PH9	20	SP3	1:75	THERMO	TJ264922	15	POLYMER	N	N	DAB
149	high pH OMNIS	20 min 97 C	SP3	1:100	Dako	20058975	20	EnVision Flex	No	No	DAB
151	HIER2/BUFFER 9.0	20	SP3	1:75	THERMO SCIENTIFIC	RL2310171	15	BOND REFINE	N	N	DAB
175	HIER	32 mins	4B5	predilute	Roche	E14806	16 mins	polymer	N	Y(copper)	DAB
181	CC1 on board	30 minutes	4B5	pre-diluted	Roche	E18754	16 minutes	Ventana Ultraview DAB	N	Y	DAB
186	HIER	20	HER2	1:400	Dako	20050631	15	Leica Bond Polymer Refine Kit	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	E16719	24	Optiview	N	N	DAB
189	CC1	32	4B5	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
194	HIER (CC1)	36	4B5	RTU	ROCHE	E13637	12	ULTRAVIEW	N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Ventana/Roche	E22618	28 min	Ultraview	N	Y	DAB
202	er1	40	Her2	rtu	DAKO Agilent	20059542	30	Herceptest kit	no	no	DAB
207	CC1- on line	36 minutes	4B5	prediluted	Ventana	E14806	16 minutes	Ultraview	N	Y	DAB
217	hier	32	4b5	rtu	ventana	e12497	20	optiview	n	y	dab
220	CC1	20 min	4B5	Pre-Dilute	Roche	E17552	12 min	UltraView	N	N	DAB
221	pH 6 Citrate	30	HER2	Neat	Dako	20060758	30	Hercept Test Buffer	N	N	DAB
230	HIER	36	4B5	PREDILUTE	Ventana	E14806	16	OPTIVIEW	N	N	DAB
233	CC1	36mins	4B5	NA	ROCHE	13637	16mins	ULTRAVIEW	N	Y	DAB

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	112	85.71	92	91/92 (99%)	0.98	1	0.98
102	112	89.29	96	95/96 (99%)	0.98	1	0.98
103	112	89.29	94	93/94 (99%)	0.98	1	0.98
106	112	88.39	93	92/93 (99%)	1	0.98	0.98
107	112	84.82	90	88/90 (98%)	0.96	1	0.96
109	112	57.14	60	57/60 (95%)	0.96	0.94	0.9
111	112	84.82	91	90/91 (99%)	0.98	1	0.98
112	112	84.82	91	90/91 (99%)	1	0.98	0.98
113	112	91.07	96	93/96 (97%)	1	0.93	0.94
114	112	86.61	92	91/92 (99%)	0.98	1	0.98
120	112	83.93	91	88/91 (97%)	0.94	1	0.93
123	112	28.57	31	31/31 (100%)	1	1	1
125	112	88.39	94	94/94 (100%)	1	1	1
127	112	83.04	88	87/88 (99%)	0.98	1	0.98
128	112	89.29	95	94/95 (99%)	0.98	1	0.98
129	112	91.96	97	95/97 (98%)	0.98	0.98	0.96
132	112	83.04	88	86/88 (98%)	0.96	1	0.95
133	112	87.5	93	92/93 (99%)	0.98	1	0.98
134	112	83.93	91	90/91 (99%)	0.98	1	0.98
138	112	85.71	91	90/91 (99%)	0.98	1	0.98
141	112	84.82	92	90/92 (98%)	0.96	1	0.96
144	112	86.61	92	92/92 (100%)	1	1	1
147	112	91.96	97	95/97 (98%)	0.96	1	0.96
148	112	85.71	91	89/91 (98%)	0.96	1	0.96
149	112	82.14	88	86/88 (98%)	0.96	1	0.95
151	112	87.5	92	91/92 (99%)	0.98	1	0.98
175	112	83.93	90	88/90 (98%)	0.98	0.98	0.96
178	112	54.46	60	58/60 (97%)	0.92	1	0.93
180	112	87.5	93	91/93 (98%)	0.98	0.98	0.96
183	112	56.25	58	57/58 (98%)	0.95	1	0.96
186	112	88.39	93	92/93 (99%)	0.98	1	0.98
187	112	91.96	97	94/97 (97%)	0.95	1	0.94
188	112	55.36	60	58/60 (97%)	0.91	1	0.93
189	112	87.5	93	92/93 (99%)	0.98	1	0.98
190	112	70.54	78	76/78 (97%)	0.95	1	0.95
192	112	86.61	92	91/92 (99%)	0.98	1	0.98
194	112	90.18	96	95/96 (99%)	0.98	1	0.98
198	112	80.36	85	82/85 (96%)	0.98	0.95	0.93
202	112	73.21	80	80/80 (100%)	1	1	1
207	112	85.71	91	90/91 (99%)	0.98	1	0.98
209	112	91.07	98	97/98 (99%)	0.98	1	0.98
217	112	56.25	60	60/60 (100%)	1	1	1
220	112	84.82	92	91/92 (99%)	0.98	1	0.98
221	112	95.54	101	98/101 (97%)	0.95	1	0.94
230	112	83.04	88	87/88 (99%)	0.98	1	0.98
233	112	85.71	91	89/91 (98%)	0.96	1	0.96

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	48	89.58	42	41/42 (98%)	0.94	1	0.95
102	48	91.67	43	42/43 (98%)	1	0.96	0.95
103	48	91.67	43	42/43 (98%)	1	0.96	0.95
106	48	91.67	43	42/43 (98%)	1	0.96	0.95
107	48	91.67	43	41/43 (95%)	1	0.92	0.91
109	48	87.5	42	42/42 (100%)	1	1	1
111	48	91.67	43	42/43 (98%)	1	0.96	0.95
112	48	85.42	41	41/41 (100%)	1	1	1
113	48	89.58	43	43/43 (100%)	1	1	1
114	48	89.58	43	43/43 (100%)	1	1	1
120	48	83.33	40	39/40 (98%)	0.94	1	0.95
123	48	87.5	42	42/42 (100%)	1	1	1
125	48	89.58	42	42/42 (100%)	1	1	1
127	48	89.58	42	41/42 (98%)	0.94	1	0.95
128	48	87.5	42	38/42 (90%)	1	0.84	0.81
129	48	91.67	43	42/43 (98%)	1	0.96	0.95
132	48	91.67	43	43/43 (100%)	1	1	1
133	48	91.67	43	43/43 (100%)	1	1	1
134	48	91.67	43	40/43 (93%)	1	0.88	0.86
138	48	91.67	43	42/43 (98%)	1	0.96	0.95
141	48	83.33	40	36/40 (90%)	1	0.83	0.8
147	48	89.58	42	42/42 (100%)	1	1	1
149	48	87.5	42	38/42 (90%)	0.76	1	0.79
151	48	89.58	42	40/42 (95%)	1	0.92	0.9
175	48	89.58	43	42/43 (98%)	1	0.96	0.95
178	48	89.58	43	41/43 (95%)	0.94	0.96	0.9
183	48	89.58	43	38/43 (88%)	1	0.8	0.77
186	48	89.58	42	41/42 (98%)	1	0.96	0.95
187	48	87.5	42	39/42 (93%)	0.94	0.92	0.85
189	48	85.42	41	35/41 (85%)	0.94	0.79	0.71
190	48	89.58	43	43/43 (100%)	1	1	1
192	48	89.58	43	42/43 (98%)	1	0.96	0.95
194	48	91.67	43	43/43 (100%)	1	1	1
196	48	91.67	43	41/43 (95%)	1	0.92	0.91
198	48	91.67	43	40/43 (93%)	0.83	1	0.85
202	48	91.67	43	42/43 (98%)	1	0.96	0.95
207	48	89.58	43	42/43 (98%)	1	0.96	0.95
220	48	91.67	43	42/43 (98%)	1	0.96	0.95
221	48	91.67	43	43/43 (100%)	1	1	1
230	48	87.5	42	42/42 (100%)	1	1	1
233	48	91.67	43	43/43 (100%)	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	Cohen's kappa
101	48	91.67	44	44/44 (100%)	1	1	1
102	48	89.58	43	42/43 (98%)	1	0.97	0.94
103	48	93.75	45	45/45 (100%)	1	1	1
106	48	91.67	44	44/44 (100%)	1	1	1
107	48	91.67	44	43/44 (98%)	1	0.97	0.94
109	48	87.5	42	42/42 (100%)	1	1	1
111	48	93.75	45	45/45 (100%)	1	1	1
112	48	87.5	42	41/42 (98%)	1	0.97	0.94
113	48	89.58	43	43/43 (100%)	1	1	1
114	48	91.67	44	44/44 (100%)	1	1	1
120	48	87.5	42	42/42 (100%)	1	1	1
123	48	89.58	43	43/43 (100%)	1	1	1
125	48	91.67	44	44/44 (100%)	1	1	1
127	48	91.67	44	44/44 (100%)	1	1	1
129	48	91.67	44	43/44 (98%)	1	0.97	0.94
133	48	91.67	44	43/44 (98%)	1	0.97	0.94
138	48	91.67	44	44/44 (100%)	1	1	1
147	48	89.58	43	42/43 (98%)	1	0.97	0.94
149	48	87.5	42	42/42 (100%)	1	1	1
151	48	91.67	44	44/44 (100%)	1	1	1
175	48	91.67	44	44/44 (100%)	1	1	1
181	48	87.5	42	42/42 (100%)	1	1	1
186	48	91.67	44	43/44 (98%)	1	0.97	0.94
187	48	89.58	43	43/43 (100%)	1	1	1
189	48	85.42	41	41/41 (100%)	1	1	1
190	48	91.67	44	44/44 (100%)	1	1	1
194	48	89.58	43	43/43 (100%)	1	1	1
198	48	89.58	43	43/43 (100%)	1	1	1
202	48	91.67	44	44/44 (100%)	1	1	1
207	48	91.67	44	43/44 (98%)	1	0.97	0.94
220	48	91.67	44	43/44 (98%)	1	0.97	0.94
221	48	89.58	43	43/43 (100%)	1	1	1
230	48	89.58	43	42/43 (98%)	1	0.97	0.94
233	48	91.67	44	44/44 (100%)	1	1	1

Table S3. IHC Status definitions.

IHC Status	Definition	clQc Proficiency Testing Performance
Optimal	The staining was considered of the highest technical quality to allow for accurate readout of the target biomarker.	PASS
Adequate	The staining was considered to be sufficient for the purpose of accurate readout of the target biomarker.	PASS
Sub-optimal	The staining was considered to be of a quality that makes readout of the test challenging, which may lead to inaccurate readout of the target biomarker.	PASS, CONDITIONALLY ¹
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	FAIL ²

1 – A one-time suboptimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".
1,2 – Please contact the clQc for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.