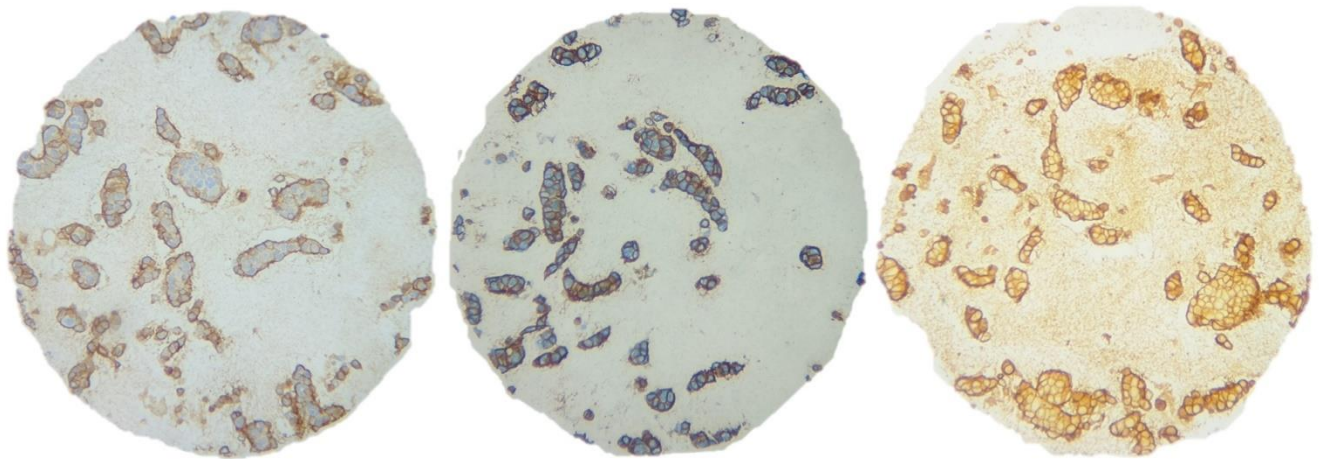


Garrattogram after cIQc assessment of HER2:

Lab ID/ Core	101	102	103	106	107	109	111	112	113	114	120	123	125	127	129	133	136	138	147	149	151	160	161	175	181	186	187	188	189	190	194	198	199	202	207	217	220	221	230	233	237	R1						
1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3			
2	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	1	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	
3	N	N	N	N	N	U	N	N	N	N	N	U	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	U	N	U	N	N	N	N	N	N	N	N	2	N		
4	N	N	N	N	N	N	N	N	N	1	N	N	N	N	1	N	N	N	1	N	1	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	2	N
5	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	3	3	3	3	3	3		
6	N	2	2	1	1	N	1	1	1	N	1	1	1	1	2	2	1	2	2	1	2	N	1	1	1	1	1	1	1	1	2	1	1	1	2	1	1	1	2	N	N	2	N	N				
7	N	N	N	N	N	1	N	N	N	N	N	N	N	N	1	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	2	N		
8	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	1	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	
9	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	3	3	3	3	3	3		
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	3	3	3	3	3	3		
11	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	1	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	
12	N	N	N	N	N	U	N	N	N	N	N	U	N	N	N	1	N	N	U	N	N	N	N	N	N	N	N	1	N	N	1	N	N	N	N	N	N	N	N	N	N	N	1	N	N	2	N	
13	N	N	1	N	N	N	N	N	N	N	N	N	N	1	1	N	N	N	1	N	1	N	N	N	N	N	N	N	N	N	1	N	N	N	1	N	N	N	N	N	N	N	N	N	2	N		
14	3	3	3	3	3	3	2	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	2	2	1	N	1	1	1	2	N	2	1	1	1	2	2	2	2	2	1	2	N	1	1	2	1	1	U	U	U	U	U	U	U	U	U	U	U	U	U	1	U	U	U	N			
16	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	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	
17	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	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18	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	3	3	3	3	3	3		



Lab 188
(Adequate; Slightly weak)

Lab 194
(Optimal)

Lab 237
(Sub-optimal; high background)

Figure 1. Representative images of HER2 staining in a 3+ core in select labs with noted staining issues.

Supplementary Tables S1-S3 provides a summary of staining protocols. Supplementary Table 4 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	EP1	RTU	Dako	10141921	20 min	DAKO Envision Flex	N	N	DAB
102	DAKO PT - HIGH PH	20	EP1	1:50	DAKO	1014617	30* RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64	SP1	PRE	VENTANA	E22622	16	ULTRA VIEW	NO	Y COPPER	DAB
106	CC1 on stainer	64	SP1	pre dilute	Roche	E27813	32	Optiview	no	yes	DAB
107	Ultra cc1	36	SP1	Pre-diluted	Ventana	E27215	16	Ultraview DAB	N	Y	DAB
109	hier high pH CC1	64 MIN	SP1	RTU	ROCHE	E27813	32 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	36	sp1	predilute	ventana	E27813	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	1/90	Invitrogen	UE2766301	21min	OPTIVIEW	N	Y	DAB
114	Envision Flex TRS, High pH	30	SP1	1:50	THERMO FISHER	9101S1803 C	30	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	HIER Waterbath	20	EP1	RTU	Dako	10136915	20	Dako Envision Flex	n	n	DAB
123	Benchmark Ultra CC1	36	SP1	predilute	Roche	E12794	32	Roche UltraView	N	N	DAB
125	HIER	30	SP1	RTU	ROCHE		30	Dako Envision Flex	Y	N	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN	SP1	PREDILUTE	VENTANA	E07983	32 MIN	ULTRAVIEW DAB	N	Y	DAB
128	Ventana Ultra CC1	64 min	SP1	Ready-To-Use	Ventana/Roche	E27813	16 min	UltraView Universal Dab Detection Kit	N	N	CuSO4
129	ER 2- high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1809A	15	Bond Refine Detection Kit	N	N	DAB
132	High pH	20	EP1	RTU	Dako/Agilent	10145592	20	Envision Flex	N	N	DAB
133	Polymer	36 Min	SP1	Pre-dilute	Ventana Roche	E22622	32 Min	Ultraview	N	N	DAB
134	HIER - CC1	30 min	SP1	RTU	VENTANA/ROCHE	E03298	8 min	ULTRAVIEW	N	N	DAB
136	DAKO PT HIGH PH	20	EP1	RTU	DAKO	10145592	20	DAKO ENVISION FLEX +	N	N	DAB
138	EDTA HIER	30	EP1	RTU	Dako	10141921	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	1803C	16 min	Opti-View	No	Copper	DAB
147	HIER pH 9	20	SP1	1:150	Thermo	9101S1803.C	15	Polymer	N	N	DAB
148	CC1	36 min	SP1	RTU	Ventana	E27813	12 min	Ultraview	NO	NO	DAB
149	high pH OMNIS	20 min at 97 C	EP1	RTU	Dako Agilent	10129810	20	EnVision Flex OMNIS	No	No	DAB
151	HIER2	20	SP1	1:50	THERMO	9101S1803 C	15	BOND REFINE	N	N	DAB
159	Flex TRS High	40 min.	EP1	RTU	Dako, Agilent	10143114	20	Dako Flex	N	N	DAB
160	CC1	36 MIN	SP1	PR ^A %-DILUA%	VENTANA	E18133	8 MIN	ULTRA-VIEW	N	Y	DAB
161	HIER-High EDTA TRIS tampon	20 Minutes	EP1	RTU	Dako	10145592	20 Minutes	Envision Flex	No	No	DAB
175	HIER	36	SP1	Pre-dilute	Roche	E27813	32	Polymer Ultra DAB	N	Y	DAB
180	CC1-32	32	SP1	RTU	VENTANA	E21981	16	OPTIVIEW	N	Y	DAB
183	Ultra CC1	36	SP1	RTU	Ventana/Roche	E27813	32	Ultraview	N	N	DAB
186	HIER	20	SP1	1:50	THERMOSCIENTIFIC	9101S1711E	15	LEICA BOND POLYMER	N	N	DAB
187	CC1	16	SP1	Predilute	Roche	E27813	8	Optiview	N	N	DAB
188	Epitope Retrieval 1	20	6F11	as per kit	Leica	60789	20	Refine Detection	N	N	DAB
189	CC1	64	SP1	RTU	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	36	SP1	PREDILUTE	VENTANA	E27813	32	VIEW	N	N	DAB
192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	E27813	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	64	SP1	RTU	ROCHE	E22622	16	ULTRAVIEW DAB	N	Y	DAB
196	n/a	n/a	sp1	no	ventana	E27813	8 minute	DAB	No	No	n/a
198	High pH HIER	30 min	EP1	Prediluted	Dako/Agilent	10145592	30 min	Envision Flex/HRP	N	N	DAB
202	BOND Epitope retrieval 1	15	6F11	1:100	Leica	6059509	16	Bond polymer Refine Detection	N	N	DAB
207	CC1-ONLINE	36 minutes	SP1	Prediluted	Ventana	E22622	16 minutes	Ultraview	N	Y	DAB
217	HIER	64	SP1	rtu	Roche Ventana	E22622	20	Optiview	N	Y	DAB
220	HIER	36 minutes	SP1	PRE DILUTE	VENTANA	E27813	36 minutes	VENTANA ULTRAVIEW	N	Y	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	E27813	32	ULTRAVIEW	N	N	DAB
233	CC1	36	SP1	NA	Ventana/Roche	E18133	16	Ultraview	N	N	DAB

Table S2. Reported PR staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, Hlgh PH	30 min	1294	RTU	Dako	10141668	25 min	DAKO Envision Flex	N	N	DAB
102	DAKO PT - HIGH PH	20	16	1:200	LEICA	6057451	30" RT	DAKO ENVISION FLEX	N	YES CUSO4	DAB+
103	CC1	64	100	PRE	VENTANA	E12265	16	ULTRA VIEW	NO	Y COPPER	DAB
106	CC1 on stainer	64	PgR 1294	1/220	Dako	10132621	32	Optiview	no	yes	DAB
107	Ultra cc1	64	PgR1294	1:50	Dako	10139537	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	6061976	32	OPTIVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	10 minutes	16	RTU	Leica	63774	15 minutes @ RT	BOND Polymer Refine	no	no	DAB
113	Dako low pH	20min	PgR636	1/250	Dako	10136680	15min	Flex+20 mouse	Y	N	DAB
114	Envision Flex TRS, High pH	30	1294	RTU	DAKO	10138258	25	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	HIER Waterbath	20	PgR 636	RTU	Dako	10134386	20	Envision Flex+	y	n	DAB
123	Benchmark Ultra CC1	64	16	1/25	Leica/Novocastra	6061976	60	Roche UltraView	N	N	DAB
125	HIER	30	PgR 1294	1/200	Dako		20	Dako Envision Flex	N	N	DAB
127	HIER	36 MIN	100	PREDILUTE	VENTANA	E09646	8 MIN	ULTRAVIEW DAB	N	Y	DAB
128	Ventana Ultra CC1	64 min	100	Ready-To-Use	Ventana/Roche	E26635	16 min	UltraView Universal Dab Detection Kit	No	No	CuSO4
129	ER 2- high pH retrieval	20	16	1:400	Novocastra	6027295	15	Bond Refine Detection Kit	N	N	DAB
132	High pH	20	PGR	1:200	Leica	6044509	30	Envision Flex	N	N	DAB
133	Polymer	36 Min	SPI	Pre-dilute	Ventana Roche	6061976	32 Min	Ultaview	N	N	DAB
134	HIER - CC1	30 min	100	RTU	VENTANA/ROCHE	E02134	12 min	ULTRAVIEW	N	N	DAB
136	DAKO PT LO PH	20	PGR 636	RTU	DAKO	10146463	20	DAKO ENVISION FLEX +	N	N	DAB
138	EDTA HIER	30	PgR 636	RTU	Dako	10142936	20	Polymer/Dako	N	N	DAB
141	HIER - CC1	30 min	IE2	RTU	VENTANA/ROCHE	E02134	12 min	ULTRAVIEW	N	N	DAB
147	HIER pH 9	20	16	1:800	NCL	60727295	15	Refine Leica Polymer	N	N	DAB
149	high pH OMNIS	20 min at 97 C	PgR1294	RTU	Dako Agilent	10130585	10	EnVision Flex OMNIS	No	No	DAB
151	HIER1	20	16	1:100	NCL	6060299	15	BOND REFINE	N	N	DAB
159	Flex TRS High	40 min.	PgR 636	RTU	Dako, Agilent	10136909	30	Dako Flex	N	N	DAB
160	CC1	36 MIN	100	PRÄ% - DILUÄ%	VENTANA	E22157	8 MIN	ULTRA-VIEW	N	Y	DAB
161	HIER-High EDTA TRIS tampon	20 Minutes	PgR 636	RTU	Dako	10146463	20 Minutes	Envision Flex	No	No	DAB
175	HIER	64	100	Pre-dilute	Roche	E26635	32	Polymer Ultra DAB	N	Y	DAB
183	Ultra CC1	36	IE2	RTU	Ventana/Roche	E26635	32	Ultraview	N	N	DAB
186	HIER	30	16	1:200	LEICA	6061976	15	LEICA BOND POLYMER	N	N	DAB
187	CC1	64	IE2	Predilute	Roche	E26635	12	Optiview	N	N	DAB
188	Epitope retrieval 2	20	16	as per kit	Leica	60927	20	Refine detection kit	N	N	DAB
189	CC1	64	100	RTU	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	36	16	1:50	LEICA	6046807	32	IVIEW	N	N	DAB
192	Ultra CC1	36 minutes	100	Ready to use	Ventana/Roche	E17507	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	64	100	RTU	ROCHE	E22157	16	ULTRAVIEW	N	Y	DAB
196	n/a	n/a	IE2	no	ventana	E17507	8 minute	dab	no	no	n/a
198	High pH HIER	30 min	1294	1/100	Dako/Agilent	10139537	20 min	Envision Flex/HRP	N	N	DAB
202	BOND Epitope retrieval 1	20	16	Ready to use	Leica	63776	16	Bond polymer Refine Detection	N	N	DAB
207	CC1 online	32 minutes	16	1/100	Leica	6061976	32 minutes	Optiview	N	Y	DAB
217	HIER	64	100	RTU	Roche Ventana	E18135	16	Otpiview	N	Y	DAB
220	HIER	36 MINUTES	100	PRE DILUTE	VENTANA	E30211	12 MINUTES	VENTANA ULTRAVIEW	N	Y	DAB
221	pH 6 Citrate	20	1A6	1:200	Leica	6052687	30	Biocare Mach IV	N	N	DAB
230	HIER	64	10	PREDILUTE	Ventana	E26635	16	ULTRAVIEW	N	N	DAB
233	CC1	48	16	1/100	LEICA	6058804	32	OPTIVIEW	N	N	DAB

Table S3. Reported HER2 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, High PH	30 min	4B5	1:8	ROCHE DIAGNOSTICS	Y28979	15 min	DAKO Envision Flex	N	N	DAB
102	DAKO PT - HIGH PH	20	SP3	1:80	CELL MARQUE	50420	30° RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	36	4B5	PRE	VENTANA	E17552	16	ULTRAVIEW	NO	Y COPPER	DAB
106	CC1 on stainer	36	4B5	pre dilute	Roche	E30207	16	Ultraview	no	yes	DAB
107	Ultra cc1	64	4B5	Pre-diluted	Ventana	E31575	24	Ultraview DAB	N	Y	DAB
109	HIER HIGH PH CC1	36 MIN	4B5	RTU	ROCHE	E27815	16 MIN	ULTRAVIEW	N	Y	DAB
111	HIER	36	4B5	PREDILUTE	VENTANA	E31575	32	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	4B5	1:4 ratio or RTU	Ventana /Roche	E14806	15 minutes @ RT	BOND Polymer Refine	no	no	DAB
113	CC1	30min	SP3	1/50	CELL MARQUE	50420	16MIN	ULTRAVIEW	N	Y	DAB
114	Envision Flex TRS, High pH	30	4B5	1:8	Roche Ventana	E11991	15	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	HIER Waterbath	40	HercepTest	RTU	Dako	20065068	30	HercepTest	n	n	DAB
123	Benchmark Ultra CC1	36	4B5	predilute	Roche	E28134	24	Roche UltraView	N	N	DAB
125	HIER	30	A0485	1/800	DAKO		10	Dako Envision Flex	N	N	DAB
127	HIER	36 MIN	4B5	PREDILUTE	VENTANA	E22628	24 MIN	ULTRAVIEW DAB	N	Y	DAB
129	ER 2- high pH retrieval	20	SP3	1:50	Thermo Scientific	TL2673411	15	Bond Refine Detection Kit	N	N	DAB
133	Polymer	36 Min	4B5	Pre-dilute	Ventana Roche	E28134	24	Ultraview	N	N	DAB
136	DAKO HERCEP TEST	40	A0485	RTU	DAKO	20065068	30	DAKO HERCEP TEST	N	N	DAB
138	HIER pH 6.0	40	HercepTest	RTU	Dako	20061666	30	HercepTest	N	N	DAB
147	HIER pH 9	20	SP3	1:75	Thermo	TJ2649422	15	Polymer	N	N	DAB
149	high pH OMNIS	20 min at 97 C	A0485	1:600	Dako Agilent	1013475	20	EnVision Flex OMNIS	no	no	DAB
151	HIER2	20	SP3	1:50	THERMO	SK2484674	15	BOND REFINE	N	N	DAB
160	CC1	36 MIN	4B5	PRÄ%--DILUÄ%	VENTANA	E27815	16 MIN	ULTRA-VIEW	N	Y	DAB
161	Herceptest epitope	40 Minutes	Rabbit anti-human HER protein	RTU	Dako	20065068	30 Minutes	Herceptest visualization reagent	No	No	Herceptest DAB chromogen
175	HIER	32	4B5	Pre-dilute	Roche	E22628	16	Polymer Ultra DAB	N	Y	DAB
181	CC1 on board	30 minutes	4B5	pre-diluted	ROCHE	E24714	16 minutes	Ventana Ultraview DAB	N	Y	DAB
186	HIER	20	POLYCLONAL	1:400	DAKO	20050631	15	LEICA BOND POLYMER	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	E28134	24	Optiview	N	N	DAB
188	Epitope Retrieval 1	25	CB11	as per kit	Leica	63957	20	Oracle IHC	N	N	DAB
189	CC1	32	4B5	RTU	Ventana	unknown	12	ultraView DAB	N	N	ultraView DAB
190	CC1	36	SP3	1:50	THERMO	9103S1701 C1	40	IVIEW	Y	N	DAB
194	CC1	36	4B5	RTU	ROCHE	E30207	12	ULTRAVIEW	N	Y	DAB
198	High pH HIER	30 min	4B5	1/5	Ventana/Roche	E24714	20 min	Envision Flex/HRP	N	N	DAB
202	ER buffer from HERCEPT test kit	40	AO485	Ready to use	Agilent	20065068	30	HERCEPT test kit	N	N	DAB
207	CC1 -ONLINE	36 minutes	4B5	Prediluted	Ventana	E30207	16 minutes	Ultraview	N	Y	DAB
217	HIER	32	4B5	RTU	Roche Ventana	E22618	20	Optiview	N	Y	DAB
220	HIER	36 MINUTES	4B5	PRE DILUTE	VENTANA	E30207	12 MINUTES	VENTANA ULTRAVIEW	N	Y	DAB
221	pH 6 Citrate	40	HER2	Neat	Dako	20065058	30	HercepTest	N	N	DAB
230	HIER	36	4B5	PREDILUTE	Ventana	E30207	16	ULTRAVIEW	N	N	DAB
233	CC1	36	4B5	NA	VENTANA/ROCHE	E22628	16	ULTRAVIEW	N	N	DAB
237	Novocastra Epitope Retrieval Solutions	30	10A7	1:100	Leica Biosystems-Novocastra	NCL-L-CBE-356	60	Novolink Polymer Detection Systems	n	n	Novolink DAB (Polymer)

Table S4. 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.