Assessors' report for cIQc Run 81: Breast Module

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Assessment performed on Monday February 5, 2018 at Dr. Everett Chalmers Regional Hospital, Fredericton NB

OVERVIEW

This run consisted of 42 cores, and was a standard breast biomarker assessment, with the tissue microarray slide stained for ER, PR and HER2. ER and HER2 staining have been of consistently high quality, across Canada, for many years and this run demonstrated this yet again. Unless labs are changing their automated immunostainer, in which case they have to revalidate, they have well-validated protocols in place that have been proven, repeatedly, to perform well based on external proficiency testing.

The advantage of the CIQC approach of using many real-life samples for each assessment is that it is possible to combine your lab's performance across multiple runs, allowing assessment of performance with a sufficiently large number of cases (n>100), that confidence intervals can be generated for the statistical parameters that are routinely assessed, i.e. sensitivity, specificity, and Kappa statistic. We do not currently do this calculation, across multiple runs (akin to the rolling average that can be used on high volume chemistry analyzers as a control) but have done it for research purposes. If you believe this would be of value to you, please let us know.

Based on the self-assessment results it was anticipated that the overall results would be good and this was born out on review. If there is a significant problem with staining for a given lab, it typically is picked up immediately, based on self-assessment, and corrected. If we pick up an egregious issue with staining in your laboratory, we will immediately contact. Only minor issues are identified, in most runs, and reported upon.

As usual, we excluded cores where there were too few labs able to obtain interpretable staining (usually as a result of there not being tissue or only a few tumor cells in the core) from the statistical analysis e.g. for ER we excluded cores 16, 19, 20, 27 and 32. Statistics provided are based on the review assessment for ER and HER2, and based on the self assessment for PR.

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.

ER

It is important to note that 100% sensitivity and specificity on EVERY CIQC ER run is impossible; the main reasons for this are 1. There are tumors with relatively few cells and variable ER expression (possibly related to variable fixation), such that the results of assessing slides cut at different levels of the TMA block will given different results, even when stained in the same lab, 2. Some tumors express very low levels of ER protein (this is especially true of basal-like breast cancers, where the number showing weak ER positivity, Allred score 3-5, is highly variable). Core 38 is an example of a core with relatively few tumor cells and heterogeneity of staining. A disadvantage of using tissue microarrays is this problem of small sample size and, because the tissue cores are from lumpectomy or mastectomy specimens, potential variability in fixation. Core 28, on the other hand, is negative in most labs in Canada, but those labs with more sensitive staining had weak but unequivocally positive staining. It is important for everyone involved in breast cancer diagnosis and treatment to recognize that weak ER positivity (Allred scores 3-5)

does not carry the same implications as the more common strong ER positivity (Allred scores 7 or 8) e.g. may be seen in basal-like breast cancers, and such cases appear to be increasing with newer more sensitive detection systems.

The upshot of the points noted above, is that there will never be 100% agreement between labs for ER staining. There is certainly agreement for tumors expressing high levels of ER protein, in that all labs in Canada consistently identify breast cancers with Allred 7 or 8 ER positivity, but there are breast cancers with low-level or equivocal ER expression where some variability in ER assessment remains. Most such tumors are high grade and/or HER2 positive, and on that basis will be candidates for adjuvant chemotherapy.

Apart from these general observations there was little noteworthy in the ER testing, with a few specific comments below, for individual laboratories. We did change a significant number of results at review, often changing results to Unsatisfactory (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%).

Lab ID	Comments
103	A number of cores that were considered positive on self-assessment were rescored as negative.
141	Relatively weak staining e.g. false negative result for core 6
221	Particularly strong nuclear counterstain, accounting for false negative results in tumors with weak ER expression e.g. cores 1, 6, 9

Lab/ Core	101	102	103	106	107	109	111	112	114	120	123	125	127	128	129	132	133	134	138	141	144	147	148	149	151	175	178	183	186	187	189	190	192	194	196	198	199	202	207	209	217	221	230	233	R1
1	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	P	P	N	P	P	N	P	P	P	N	N	N	N	N	N	N	N	N	P	P	N	N	P	P
2	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P
3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4	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	N	N	N	N
5	P	U	P	P	P	P	U	U	P	P	P	P	P	P	P	U	P	P	U	P	P	P	P	U	P	U	U	P	U	U	U	P	P	U	U	U	U	U	P	P	P	P	U	P	P
6	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	N	P	P	P	P	N	P	P	P
7	U	U	U	P	P	U	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U	P	P	P	U	P	P	P	P	P	P	U	U	P	P	P	U	U	P	U	U	P
8	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
9	P	P	P	P	P	P	U	U	P	P	U	P	P	P	P	U	P	U	P	P	U	U	U	U	U	P	U	P	U	U	P	U	P	P	P	P	N	U	P	P	P	N	U	U	P
10	U	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	U	U	U	U	U	P	U	U	P	U	U	U	P	U	P	P
11	P	P	P	P	P	P	U	U	U	U	U	P	U	P	P	P	P	U	U	U	U	U	U	U	P	P	U	P	P	P	P	P	P	P	P	U	P	U	P	P	U	P	U	U	P
12	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
13	U	U	P	P	P	U	P	P	P	U	P	P	P	U	P	P	P	U	P	P	P	P	U	P	P	P	U	P	P	P	P	P	P	P	P	P	P	U	P	P	P	U	P	P	P
14	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P
15	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
17	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
18	P	P	P	P	P	P	P	U	U	P	U	P	P	P	P	U	P	U	U	P	U	U	P	U	U	P	U	U	U	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P
21	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
22	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U	P	P
23	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
24	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	N	N	N	N
25	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P
26	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
28	N	N	N	P	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	N	N
29	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	N	N	N	N
30	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	U	N	N	N	N	N	N	N	N	N
31	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
33	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	N	N	U	N
34	U	U	U	P	N	U	N	U	N	N	N	P	N	N	U	N	N	N	U	N	N	N	N	N	N	N	N	N	N	U	N	U	N	U	N	U	N	U	U	U	U	U	U	U	N
35	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
36	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	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N
37	P	N	N	P	N	P	N	N	P	N	N	P	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	P	N	U	N
38	N	U	N	N	N	U	N	U	U	U	U	U	N	N	U	U	N	N	U	N	P	U	N	N	N	P	N	P	P	N	N	N	N	N	N	N	N	U	N	N	U	N	N	U	N
39	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P
40	P	U	P	U	U	P	P	U	P	U	U	U	U	U	U	U	U	U	U	P	P	P	U	U	U	U	U	U	P	U	P	P	P	P	P	N	U	P	P	P	P	U	U	P	P
41	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	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
42	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P

*Cores 16, 19, 20, 27, and 32 were excluded from all analyses.

HER2

After exclusion of technically unsatisfactory cores (e.g. Cores 16, 19, 20 and 32) and those in which invasive carcinoma was not present on most slides (e.g. Core 9), as well as assessment review where, for example, Core 36 was changed from 3+ to 2+ by the reviewing team, there were no false positive or false negative results. Congratulations!

Lab/ Core	102	103	106	107	109	111	112	114	120	125	127	129	133	138	147	149	151	175	181	186	187	189	190	194	198	199	202	207	217	221	230	233	FISH
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	3	3	3	3	3	Amp
2	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	Non-amp
3	1	1	N	N	N	N	N	N	U	N	1	N	N	U	N	N	N	N	N	1	N	N	N	N	N	N	N	N	1	1	N	N	Non-amp
4	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	Amp
5	3	3	3	U	U	U	3	3	U	3	3	U	3	3	3	U	3	3	3	3	U	3	3	U	U	U	U	3	3	2	U	3	Amp
6	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	Non-amp
7	U	U	U	U	U	U	N	N	N	N	N	N	U	U	U	U	U	U	N	1	U	N	1	U	U	N	U	N	U	U	U	N	Non-amp
8	1	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	1	N	N	N	N	N	Non-amp
10	U	N	U	N	U	N	N	N	N	N	N	N	N	U	N	N	N	N	N	1	U	U	U	U	U	U	U	U	U	N	U	N	Non-amp
11	1	2	1	U	U	U	U	U	U	U	U	N	U	U	N	U	1	1	U	2	1	N	N	1	1	N	U	1	1	2	N	N	Non-amp
12	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	Non-amp
13	1	U	N	N	N	U	N	N	1	U	N	U	U	U	U	U	1	N	N	2	N	N	N	N	U	N	U	N	1	1	N	U	Non-amp
14	3	2	2	2	2	2	2	3	2	2	2	3	2	2	3	3	3	2	2	3	2	2	3	2	2	3	2	2	2	2	2	2	Amp
15	2	2	1	N	1	N	1	N	1	N	1	1	1	1	1	N	1	N	N	1	N	N	N	N	N	N	1	N	1	1	1	N	Non-amp
17	N	1	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	Non-amp
18	N	1	N	U	N	N	U	U	U	N	N	N	N	U	N	U	U	N	U	U	U	N	N	N	N	N	N	N	N	N	N	N	Non-amp
21	N	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	1	N	N	1	N	N	N	N	N	N	N	N	N	Non-amp
22	1	2	1	N	1	1	U	N	1	N	1	U	N	2	1	N	N	N	N	2	N	N	1	N	N	N	2	N	1	1	1	N	Non-amp
23	1	2	N	1	1	N	U	N	2	1	1	N	1	2	1	N	N	2	2	2	1	N	N	N	N	N	2	N	N	1	N	1	Non-amp
24	3	2	2	3	2	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	2	2	3	2	2	3	3	2	2	3	3	2	Amp
25	N	N	N	N	N	N	U	N	U	N	N	U	N	U	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	Non-amp
26	1	1	N	1	N	N	1	N	1	N	N	1	1	1	1	1	1	1	N	2	N	N	N	N	N	1	2	N	1	1	N	N	Non-amp
27	U	N	U	U	N	U	U	U	N	U	U	N	N	U	N	U	U	U	U	N	U	N	N	U	U	N	U	U	U	N	U	U	Non-amp
28	2	2	1	1	1	N	1	N	1	N	1	N	2	1	2	N	N	2	1	2	N	N	1	N	1	1	2	1	1	1	1	N	Non-amp
29	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	Amp
30	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	Non-amp
31	1	1	N	N	N	N	N	N	1	N	N	1	1	N	N	N	N	N	N	1	N	N	N	N	N	N	N	N	N	1	N	N	Non-amp
33	3	3	3	2	2	2	3	2	3	2	3	3	3	3	2	3	2	3	3	3	3	2	2	3	3	3	2	3	3	3	3	3	Amp
34	2	2	U	1	1	1	N	N	U	1	1	2	1	U	1	1	1	N	1	2	U	N	1	U	U	U	N	N	N	1	1	U	N	Non-amp
35	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	Non-amp
36	2	2	1	1	1	1	2	2	2	1	1	2	2	2	2	2	2	2	2	2	1	N	1	1	1	1	2	1	1	2	1	1	Non-amp
37	3	3	3	2	2	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	2	2	3	3	3	3	3	2	2	3	3	3	Amp
38	3	3	3	3	U	3	U	U	U	U	3	U	3	U	3	3	2	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	Amp
39	2	2	1	1	1	1	N	1	1	1	1	1	2	1	1	1	2	1	2	1	N	1	1	1	N	2	1	1	1	1	1	Non-amp
40	U	N	U	U	U	U	N	N	U	U	U	N	U	U	N	U	U	U	U	U	N	N	1	N	N	N	U	U	N	N	U	N	Non-amp
41	1	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N	N	U	N	1	N	N	N	N	N	N	1	N	U	N	N	N	Non-amp
42	1	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	U	N	N	N	Non-amp

*Cores 9, 16, 19, 20, and 32 were excluded from all analyses.

PR

Quality assurance/external proficiency testing for PR remains problematic, as there is no accepted "gold standard" for comparison. It is a mystery how such an inferior biomarker remains widely used in practice. In talking to clinicians some have suggested that the main "use" of PR is to look for PR+/ER- tumors, as a flag for a false negative ER stain. We are aware of no data to support this, and suspect that this practice relates to experiences from 15 or more years ago, when ER staining was not of the uniformly good quality that it is now. In summary, there is considerable variability in PR staining results, and this is both predictable and will not change for the foreseeable future. Some of us continue to nurse hopes that this biomarker can be dropped someday.

The statistical evaluation of results performed by CIQC gives results that reflect the variability in staining between labs and our inability to know which result is correct, so that there is no way to improve uniformity of staining between labs. The statistical measures of performance reflect this in showing less

agreement with "reference" values than for ER or HER2. Whatever. Given that, as noted previously, we don't have a reliable reference result for PR, we advise against attempting to remediate any results but those that are outliers compared to the rest of Canada. Based on the self assessment results four labs (103, 128, 175 and 187) stood out as having positive PR staining in cases where most other labs did not, resulting in lack of specificity when statistics were calculated based on the self assessment results. This was especially noteworthy as some of these cases were ER negative (consistently so, across Canada) and the ER-/PR+ phenotype should be rare. When reviewed independently, there were a significant number of the cores reported as positive that we considered to be negative on review. This was due to positive staining of benign, non-tumor cells, for example. After review, the results of all four labs improved, especially lab 103, but also labs 175 and 187. Lab 128 had very weak staining of the nuclei (a blush, really) in a number of ER negative tumors (cores 28, 33 and 41), and it is likely that this is non-specific staining.

Lab/ Core	101	102	103	106	107	109	111	112	114	120	125	127	128	129	132	133	134	138	141	147	149	151	175	178	183	186	187	189	190	192	194	196	198	199	202	207	209	217	221	230	233	R1
1	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N	P	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N	P	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
3	P	P	P	P	P	P	P	N	N	P	N	N	P	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	N	N	U	P	P	P	P	N	P	P	N	U	N	P
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N
5	U	N	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	U	N	N	U	N	N	U	U	N	U	P	U	N	U	N	U	U	U	N	N	U	N	U	N	N
6	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
7	P	U	U	U	P	U	U	P	P	P	P	P	U	P	U	P	P	P	P	P	U	P	U	P	U	P	P	P	U	P	U	U	U	P	P	P	U	U	P	U	P	P
8	P	P	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
9	U	N	N	N	N	N	U	U	U	N	N	N	P	N	P	P	U	N	P	U	P	P	P	N	P	U	U	P	N	N	N	P	N	N	U	N	N	N	N	U	N	N
10	N	P	P	P	P	U	P	U	P	N	U	N	P	P	P	P	P	U	P	P	P	P	P	P	P	N	U	U	U	N	U	N	U	U	U	U	U	U	U	U	N	P
11	P	P	P	P	P	P	U	U	U	P	U	U	P	U	U	N	N	U	N	U	U	P	P	U	P	P	P	P	P	P	P	U	P	P	P	P	P	P	P	U	N	P
12	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
13	P	U	P	P	P	U	U	P	P	P	P	P	P	P	U	P	U	P	U	P	P	P	P	U	P	P	P	P	U	P	P	P	P	P	P	P	U	P	P	P	U	P
14	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
15	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
16	U	U	N	U	U	U	U	U	U	U	U	U	N	N	U	U	U	U	N	U	U	U	U	N	U	N	U	N	U	N	U	N	U	U	U	U	U	U	U	U	N	N
17	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	N	N	N	N	N	N	N	N
18	U	P	P	P	P	P	P	U	U	P	P	P	P	P	U	N	U	U	U	U	U	U	P	N	U	U	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
19	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	N	U	N	U	U	U	U	U	U	U	U	U	N
20	U	U	N	U	U	U	U	U	U	U	U	U	N	N	U	U	U	U	U	U	U	U	U	U	U	N	U	N	U	N	U	N	U	U	U	U	U	U	U	U	N	N
21	N	N	N	N	N	N	N	N	N	N	N	N	P	N	P	P	N	N	N	P	N	N	P	N	P	N	N	P	N	N	N	N	N	N	U	N	N	P	N	P	N	N
22	N	P	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	U	N	N	N	N	P	N	N	N	N	N	N
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
24	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	N	N
25	P	P	P	P	P	N	P	U	P	P	U	N	N	N	P	P	N	U	N	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	N	P
26	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
27	U	U	N	U	U	U	U	U	U	P	U	U	U	P	U	P	U	U	U	P	U	U	P	U	U	N	U	N	U	P	U	N	U	U	U	U	N	U	U	U	U	P
28	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N
29	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	N	N	N	N	N	N	N	N
30	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	U	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N
31	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
32	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N	U	U	U	N	U	U	U	N	U	U	U	U	U	U	U	U	U	U	U	N
33	N	P	P	N	N	N	N	N	N	N	N	N	P	N	P	N	P	N	N	N	N	N	P	N	P	N	P	N	N	N	N	P	N	N	N	N	P	N	P	N	N	N
34	N	U	U	N	N	N	N	N	N	N	N	N	N	N	U	N	N	U	N	N	N	N	N	N	N	N	U	N	N	P	U	U	U	U	U	N	U	U	N	U	N	N
35	N	P	P	P	P	P	P	N	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	N	P	N	P	P	N	P	N	P	N	N	P	N	P	P	P	P	N	P
36	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	N
37	N	P	N	N	P	N	N	N	N	P	N	N	N	P	P	N	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N
38	N	N	N	N	U	N	N	U	U	U	U	N	N	N	U	N	N	U	N	N	N	N	U	N	P	N	P	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N
39	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
40	U	P	P	P	P	U	P	U	P	P	U	U	U	N	U	U	N	U	U	P	U	U	U	U	U	N	P	U	N	P	P	P	P	P	P	P	P	P	U	N	P
41	N	N	P	N	N	N	N	N	U	N	N	N	P	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	N	N
42	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P

Supplementary Tables 1-3 summarizing staining protocols and Supplementary Tables 4-6 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. Your participation in cIQc is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Table S1. Reported ER staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 min	SP1	1:50	THERMO FISHER	9101S1704E	32min	OptiView	N	Y	DAB
102	DAKO PT - HIGH PH	20	EP1	1:50	DAKO	10126585	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64 mins	SP1	Pre	Ventana	G10362	16 mins	ultra view	N	Y copper	dab
106	CC1	64	SP1	predilute	Roche	Y07227	32	Optiview	no	yes	DAB
107	ultra cc1	36	SP1	Pre-diluted	Ventana	Y05956	16	Ultraview DAB	N	Y	DAB
109	HIER high pH (CC1)	64 MIN	SP1	RTU	ROCHE	Y03212	32 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	36	SP1	PREDILUTE	VENTANA	Y3566	32	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	SP1	1:200	ThermoFisher	9101S11604E	15 minutes @ RT	BOND polymer refine	no	no	DAB
114	CC1	32	SP1	1/50	Thermo Fisher	9101S1604K	16	Optiview	N	Y	DAB
120	waterbath (TRS High)	20	EP1	RTU	Dako	10120130	20	Envision Flex+	N	N	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN	SP1	PREDILUTE	VENTANA	G05134	32 MIN	ULTRAVIEW DAB	N	N	DAB
128	CC1	64 min	SP1	Pre-dilute	Ventana	Y07227	16 min	Ultraview	No	No	DAB
129	ER 2- high pH retrieval	20	SP1	1:50	Thermo Scientific	9101S1704D	15	Bond Refine Detection Kit	N	N	DAB
132	high pH heat	20	EP 1	RTU	DAKO	10123746	20	Envision Flex	n	n	DAB
133	HEIR	36	SP1	predilute	Roche	F09522	32	polymer- Ultraview	n	n	Dab
134	CC1 - HIER	30 min	SP1	RTU	Ventana/Roche	G10362	8 min	Ultraview	N	N	DAB
138	HIER High pH	30	EP1	RTU	Dako	10119964	20	Dako Envision Flex (Omnis)	N	N	DAB
141	HIER-CC1	30	SP1	RTU	VENTANA/ROCHE	G10362	8	ULTRAVIEW	N	N	DAB
144	CC1	24 min.	SP1	1:50	ThermoScientific	1704D	16 min.	Opti-View	No	Copper	DAB
147	ER-2	20 min	SP1	1:50	Thermo	9101S1704D	15 min	Bond Polymer Refine	N	N	DAB
148	CC1	36 min	SP1	RTU	Ventana	Y07227	12 min	Ultraview	no	no	DAB
149	PT Link high pH	20 min at 97 C	EP1	RTU	Dako Agilent	10125535	20	EnVision Flex	No	No	DAB
151	BUFFER PH 9.0	20 MIN	SP1	1:50	THERMO FISHER	9101S1704D	15 MIN	BOND REFINE	N	N	DAB
175	hier	36 minutes	SP1	Predilute	Roche	407227	32 minutes	polymer	N	y-copper	..
178	HIER	32	SP1	none	Ventana	G08481	16	Ultraview	n	n	DAB
183	ULTRA CC1	36	SP1	RTU	VENTANA	Y03212	32	ULTRAVIEW	N	N	DAB
186	HIER	20	SP1	1:50	THERMOSCIENTIFIC	9101S1704D	15	LEICA BOND POLYMER	N	N	DAB
187	CC1	16	SP1	None	Roche	Y03212	8	Optiview	N	N	DAB
189	CC1	64	SP1	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	SP1	Predilute	Ventana	Y07227	32	iView DAB	N	N	DAB
192	Ultra CC1	36 minutes	SP1	Ready to use	Ventana/Roche	Y07227	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	SP1	Predilute	Roche/Ventana	Y05956	12	IVIEW	N	Y	DAB
196			SP1		Ventana	Y03212	8 min	DAB			
198	Envision Flex Tris high pH	30 min	EP1	Prediluted	Dako/Agilent	10125535	17 min	Envision Flex/ HRP	N	N	DAB
199	HIER (ER-1)	20	6F11	Predilute	Lecia	48652	15	Bond Refine (Polymer)	N	N	DAB
202	er1	20	6f11	1/150	novocastra/leica	6046810	16	Refine Detection system	n	n	dab
207	CC1- on line	36 minutes	SP1	pediluted	Ventana	Y05956	16 minutes	Ultraview	N	Y	DAB
209	20 min at 97C and 20 min cooling down to 85C	20	EP1	Prediluted	Dako	10127410	30	Dako EnVision Flex+	N	N	DAB
217	CCL1	64	SP1	predilute	Roche Ventana	G10362	20	Optiview	No	No	DAB
221	pH6 Citrate Buffer	20 Minutes	SP1	1:100	Cell Marque	1617304D	30 Minutes	Rabbit EnVision	N	N	DAB
230	HIER	64 mins	SP1	Predilute	Ventana	Y13566	32 mins	Optiview	N	N	DAB
233	CC1	36	SP1	NA	Roche	Y07227	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	CC1	32 min	16	1:100	Novocastra	6027462	32min	Optiview	N	Y	DAB
102	DAKO PT - HIGH PH	20	16	1:250	NOVOCASTRA	6044509	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64 mins	1E2	Pre	Ventana	G05674	16 mins	ultra view	N	Y Copper	DAB
106	CC1	64	PgR1294	1:220	Dako	10117646	32	Optiview	no	yes	DAB
107	ultra cc1	64	PqR1294	1:50	Dako	10125926	32	Ultraview DAB	Y	Y	DAB
109	HIER high pH (cc1)	36 min	PgR 1294	1/50	DAKO	10114753	16 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	32	16	1:80	LEICA	6406807	32	OPTIVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	10 minutes	16	RTU	Leica	51380	15 minutes	BOND polymer refine	no	no	DAB
114	CC1	32	16	1/25	Leica	6046807	16	Optiview	N	Y	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN	1E2	PREDILUTE	VENTANA	G04962	8 MIN	ULTRAVIEW DAB	N	N	DAB
128	CC1	64 min	1E2	Pre-dilute	Ventana	Y06097	16 min	Ultraview	No	No	DAB
129	ER 2- high pH retrieval	20	16	1:400	Novocastra	6027295	15	Bond Refine Detection Kit	N	N	DAB
132	high ph heat	20	16	1:200	Leica	6044509	30	Envision Flex	n	n	DAB
133	HEIR	64 minutes	16	1/25	Leica	6046807	60	polymer- Ultraview	n	n	Dab
134	CC1 - HIER	30 min	1E2	RTU	VENTANA/ROCHE	G04962	12 min	Ultraview	N	N	DAB
138	HIER High pH	30	1294	RTU	Dako	10121715	20	Dako Envision Flex (Omnis)	N	N	DAB
141	HIER-CC1	30	1E2	RTU	VENTANA/ROCHE	G04962	12	ULTRAVIEW	N	N	DAB
147	ER2	20	16	1:800	NCL	6027295	15	Bond Polymer Refine	N	N	DAB
149	PT Link high pH	20 min at 97 C	PgR636	RTU	Dako Agilent	10122270	20	EnVision Flex	Yes	No	DAB
151	BUFFER PH 6.0	20 MIN	1A6	1:200	NCL	6027295	15 MIN	BOND REFINE	N	N	DAB
175	hier	64 minutes	1E2	Prediluet	Roche	Y06097	32 minutes	polymer	N	Y-copper	..
178	HIER	32	1E2	none	Ventana	F09460	16	Ultraview	N	N	DAB
183	ULTRA CC1	36	1E2	RTU	VENTANA	G08195	32	ULTRAVIEW	N	N	DAB
186	HIER	20	PR88	1:100	BIOGENEX	MU3281215	15	LEICA BOND POLYMER	N	N	DAB
187	CC1	64	IE2	Predilute	Roche	Y03229	12	Ultraview	N	N	DAB
189	CC1	64	1E2	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	16	1:50	Novocastra	6041139	32	iView DAB	N	N	DAB
192	Ultra CC1	36 minutes	1E2	Ready to use	Ventana/Roche	Y03229	16 minutes	Ventana Ultraview DAB	N	Y (copper)	DAB
194	CC1	30	1E2	Predilute	Roche/Ventana	G09134	20	IVIEW	N	Y	DAB
196			1E2		Ventana	y03229	8 min	DAB			
198	Envision Flex Tris high pH	30 min	1294	1/100	Dako/Agilent	10117646	20 min	Envision Flex/ HRP	N	N	DAB
199	HIER (ER-2)	20	16	1:200	Lecia	6046807	15	Bond Refine (Polymer)	N	N	DAB
202	ER2	30min	1.60E	rtu	leica	60008	16	Refine Detection system	n	n	DAB
207	CC1- on line	64	16	1/50	Vector	6031757	32minutes	Ultraview	N	Y	DAB
209	20 min at 97C and 20 min cooling down to 85C	20	PgR636	Prediluted	Dako	10122270	20	Dako EnVision Flex+	Y	N	DAB
217	CCL1	64	IE1	predilute	Roche Ventana	Y00807	16	Optiview	No	No	DAB
221	pH6 Citrate Buffer	20 Minutes	1A6	1:200	Leica	6046807	30 Minutes	Mouse EnVision	N	N	DAB
230	HIER	64	1E2	None	Ventana	Y06097	16 mins	Optiview	None	N	DAB
233	CC1	48	16	1/100	Leica	6041139	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
102	DAKO PT - HIGH PH	20	SP3	1:50	THERMO FISHER	9103S1701A	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	36	4B5	pre	Ventana	G09203	16 mins	Ultra View	N	Y copper	DAB
106	CC1	36	4B5	predilute	Roche	Y07151	16	Ultraview	no	yes	DAB
107	ultra cc1	64	4B5	Pre-diluted	Ventana	Y08422	24	Ultraview DAB	N	Y	DAB
109	HIER high pH (cc1)	36 min	4B5	RTU	ROCHE	Y07151	16 MIN	ULTRAVIEW	N	Y	DAB
111	CC1	36	4B5	PREDILUTE	VENTANA	Y14374	32	ULTRAVIEW	N	Y	DAB
112	BOND ER2 pH 9.0	20 minutes	4B5	1:4 ratio of the RTU	Ventana/Roche	G09203	15 MINUTES @ RT	BOND polymer refine	no	no	DAB
114	CC1	32	SP3	1/200	Thermo Fisher	9103S1509	32	Optiview	N	Y	DAB
120	Waterbath (HercepTest)	40	Her2 Antibody	RTU	Dako	20044033	30	HercepTest	N	N	DAB
127	HIER (BENCHMARK ULTRA)	36 MIN	4B5	PREDILUTE	VENTANA	Y07151	24 MIN	ULTRAVIEW DAB	N	N	DAB
129	ER 2- high pH retrieval	20	SP3	1:100	Thermo Scientific	RL2310171	15	Bond Refine Detection Kit	N	N	DAB
133	HEIR	36	4B5	predilute	Roche	G07935	24	polymer- Ultraview	n	n	dab
138	HIER	40	HercepTest (AO485)	RTU	Dako	20048070	30	HercepTest	BN	N	DAB
147	ER-2	20	SP3	1:100	Thermo Fisher	SA2327511	15	Bond Polymer Refine	N	N	DAB
149	PT Link high pH	30 min at 97 C	SPC3 Mono R	1:100	Thermo RM4103-S	00S1701A	20	EnVision Flex	Yes	No	DAB
151	BUFFER PH 9.0	20 MIN	SP3	1:75	THERMO	RK2291861	15 MIN	BOND REFINE	N	N	DAB
175	hier	32 minutes	4B5	predilute	Roche	410418	16 minutes	polymer	N	y-copper	..
181	CC1	30 minutes	4B5	pre-diluted	Ventana/Roche	G04794	16 minutes	Ventana Ultraview DAB	no	yes	DAB
186	HIER	20	POLYCLONAL	1:400	DAKO	20023582	15	LEICA BOND POLYMER	N	N	DAB
187	CC1	16	4B5	Predilute	Roche	Y08422	24	Optiview	N	N	Dab
189	CC1	32	4B5	pre-dilute	Ventana	unknown	16	ultraView DAB	N	N	ultraView DAB
190	CC1	32	SP3	1:50	Thermofisher	9103S10606B	40	iView DAB	N	N	DAB
194	CC1	30	4B5	Predilute	Roche/Ventana	Y10418	12	IVIEW	N	Y	DAB
198	CC1	36 min	4B5	Prediluted	Ventana/Roche	Y11029	32 min	Ultraview	N	Y	DAB
199	HIER (ER-2)	20	SP3	1:300	Cell Marque	1602506A	15	Bond Refine (Polymer)	N	N	DAB
202	Herceptest kit	40	Her2	RTU	DAKO	20048566	30	Herceptest kit	n	n	dab
207	CC1-on line	36 minutes	4b5	prediluted	Ventana	Y10418	16 minutes	ultraview	N	Y	DAB
217	CCL1	32	4B5	predilute	Roche Ventana	Y08422	20	Optiview	No	No	DAB
221	Dako Visualization Solution	40	Dako Hercep Test	NEAT	Dako	20048050	30 Minutes	Dako Kit	N	N	DAB
230	HIER	36 mins	4b5	Predilute	Ventana	Y12085	16 mins	Optiview	N	N	DAB
233	CC1	36	4B5	NA	Roche	Y04603	16	Ultraview	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	Cohen's kappa
101	37	89.19	33	32/33 (97%)	1	0.9	0.93
102	37	83.78	31	31/31 (100%)	1	1	1
103	37	94.59	35	35/35 (100%)	1	1	1
106	37	97.3	36	33/36 (92%)	1	0.73	0.79
107	37	97.3	36	35/36 (97%)	0.96	1	0.94
109	37	86.49	32	31/32 (97%)	1	0.89	0.92
111	37	91.89	34	34/34 (100%)	1	1	1
112	37	78.38	29	29/29 (100%)	1	1	1
114	37	89.19	33	32/33 (97%)	1	0.89	0.92
120	37	89.19	33	33/33 (100%)	1	1	1
123	37	86.49	32	32/32 (100%)	1	1	1
125	37	94.59	35	33/35 (94%)	1	0.8	0.85
127	37	94.59	35	35/35 (100%)	1	1	1
128	37	94.59	35	35/35 (100%)	1	1	1
129	37	91.89	34	34/34 (100%)	1	1	1
132	37	86.49	32	32/32 (100%)	1	1	1
133	37	97.3	36	36/36 (100%)	1	1	1
134	37	86.49	32	31/32 (97%)	0.95	1	0.93
138	37	78.38	29	29/29 (100%)	1	1	1
141	37	94.59	35	33/35 (94%)	0.92	1	0.87
144	37	91.89	34	33/34 (97%)	1	0.91	0.93
147	37	89.19	33	33/33 (100%)	1	1	1
148	37	89.19	33	33/33 (100%)	1	1	1
149	37	83.78	31	30/31 (97%)	0.95	1	0.93
151	37	91.89	34	34/34 (100%)	1	1	1
175	37	91.89	34	33/34 (97%)	1	0.9	0.93
178	37	75.68	28	26/28 (93%)	0.89	1	0.85
183	37	91.89	34	33/34 (97%)	1	0.91	0.93
186	37	89.19	33	32/33 (97%)	1	0.91	0.93
187	37	89.19	33	33/33 (100%)	1	1	1
189	37	91.89	34	33/34 (97%)	0.96	1	0.93
190	37	91.89	34	33/34 (97%)	0.96	1	0.93
192	37	97.3	36	35/36 (97%)	0.96	1	0.94
194	37	91.89	34	33/34 (97%)	0.96	1	0.93
196	37	94.59	35	34/35 (97%)	0.96	1	0.94
198	37	86.49	32	31/32 (97%)	0.96	1	0.93
199	37	94.59	35	27/35 (77%)	0.67	1	0.56
202	37	78.38	29	28/29 (97%)	0.95	1	0.92
207	37	94.59	35	34/35 (97%)	0.96	1	0.93
209	37	86.49	32	32/32 (100%)	1	1	1
217	37	89.19	33	33/33 (100%)	1	1	1
221	37	97.3	36	32/36 (89%)	0.88	0.9	0.74
230	37	81.08	30	29/30 (97%)	0.95	1	0.93
233	37	70.27	26	26/26 (100%)	1	1	1

Table S5. Descriptive statistics for PR based on self-assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	42	80.95	34	31/34 (91%)	0.81	1	0.82
102	42	80.95	34	30/34 (88%)	0.94	0.83	0.77
103	42	90.48	38	35/38 (92%)	0.94	0.9	0.84
106	42	85.71	36	35/36 (97%)	0.94	1	0.94
107	42	85.71	36	34/36 (94%)	0.94	0.94	0.89
109	42	78.57	33	31/33 (94%)	0.86	1	0.87
111	42	76.19	32	31/32 (97%)	0.93	1	0.94
112	42	71.43	30	27/30 (90%)	0.77	1	0.79
114	42	76.19	32	30/32 (94%)	0.88	1	0.88
120	42	83.33	35	29/35 (83%)	0.78	0.88	0.66
125	42	76.19	32	30/32 (94%)	0.86	1	0.87
127	42	83.33	35	31/35 (89%)	0.75	1	0.77
128	42	88.1	37	31/37 (84%)	0.94	0.76	0.68
129	42	92.86	39	35/39 (90%)	0.83	0.95	0.79
132	42	69.05	29	24/29 (83%)	0.92	0.75	0.66
133	42	88.1	37	32/37 (86%)	0.83	0.89	0.73
134	42	80.95	34	29/34 (85%)	0.75	0.94	0.7
138	42	64.29	27	25/27 (93%)	0.92	0.93	0.85
141	42	83.33	35	30/35 (86%)	0.8	0.9	0.71
147	42	83.33	35	32/35 (91%)	0.94	0.89	0.83
149	42	76.19	32	30/32 (94%)	0.93	0.94	0.87
151	42	85.71	36	34/36 (94%)	0.94	0.95	0.89
175	42	80.95	34	29/34 (85%)	0.94	0.76	0.71
178	42	78.57	33	29/33 (88%)	0.73	1	0.75
183	42	78.57	33	24/33 (73%)	1	0.5	0.48
186	42	92.86	39	33/39 (85%)	0.72	0.95	0.69
187	42	76.19	32	28/32 (88%)	0.94	0.8	0.75
189	42	90.48	38	33/38 (87%)	0.88	0.86	0.74
190	42	78.57	33	28/33 (85%)	0.67	1	0.69
192	42	100	42	38/42 (90%)	0.84	0.96	0.81
194	42	76.19	32	30/32 (94%)	0.87	1	0.87
196	42	88.1	37	32/37 (86%)	0.82	0.9	0.73
198	42	76.19	32	30/32 (94%)	0.88	1	0.88
199	42	80.95	34	32/34 (94%)	0.88	1	0.88
202	42	73.81	31	30/31 (97%)	0.94	1	0.94
207	42	85.71	36	32/36 (89%)	0.76	1	0.77
209	42	80.95	34	30/34 (88%)	0.88	0.89	0.76
217	42	78.57	33	28/33 (85%)	0.94	0.76	0.7
221	42	85.71	36	34/36 (94%)	0.88	1	0.89
230	42	66.67	28	25/28 (89%)	0.92	0.87	0.79
233	42	88.1	37	30/37 (81%)	0.59	1	0.61

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
102	37	89.19	33	33/33 (100%)	1	1	1
103	37	94.59	35	35/35 (100%)	1	1	1
106	37	86.49	32	32/32 (100%)	1	1	1
107	37	83.78	31	31/31 (100%)	1	1	1
109	37	83.78	31	31/31 (100%)	1	1	1
111	37	83.78	31	31/31 (100%)	1	1	1
112	37	81.08	30	30/30 (100%)	1	1	1
114	37	86.49	32	32/32 (100%)	1	1	1
120	37	78.38	29	29/29 (100%)	1	1	1
125	37	86.49	32	32/32 (100%)	1	1	1
127	37	91.89	34	34/34 (100%)	1	1	1
129	37	86.49	32	32/32 (100%)	1	1	1
133	37	89.19	33	33/33 (100%)	1	1	1
138	37	67.57	25	25/25 (100%)	1	1	1
147	37	94.59	35	35/35 (100%)	1	1	1
149	37	81.08	30	30/30 (100%)	1	1	1
151	37	89.19	33	33/33 (100%)	1	1	1
175	37	89.19	33	33/33 (100%)	1	1	1
181	37	89.19	33	33/33 (100%)	1	1	1
186	37	94.59	35	35/35 (100%)	1	1	1
187	37	83.78	31	31/31 (100%)	1	1	1
189	37	97.3	36	36/36 (100%)	1	1	1
190	37	97.3	36	36/36 (100%)	1	1	1
194	37	86.49	32	32/32 (100%)	1	1	1
198	37	83.78	31	31/31 (100%)	1	1	1
199	37	91.89	34	34/34 (100%)	1	1	1
202	37	81.08	30	30/30 (100%)	1	1	1
207	37	91.89	34	34/34 (100%)	1	1	1
217	37	86.49	32	32/32 (100%)	1	1	1
221	37	97.3	36	36/36 (100%)	1	1	1
230	37	83.78	31	31/31 (100%)	1	1	1
233	37	94.59	35	35/35 (100%)	1	1	1