Report for cIQc Run 27: Immunopanels for basal-like breast carcinoma (ER, PR, HER2, CK5 and optional biomarkers)

OVERVIEW

The purpose of Run 27 was to assess the performance of clinical laboratories in staining surrogate immunopanels for identification of basal-like breast cancer, using a duplicate core tissue microarray containing 40 breast cancer cases. Basal-like breast cancer was originally identified based on gene expression profiling studies more than a decade ago. It is a subtype of considerable interest as it behaves differently than usual luminal-type breast cancers, shows considerable differences in frequency among different ethnic groups, and is associated with BRCA1 mutations. Currently, basal-like breast carcinomas can only be reliably identified using molecular assays, such as the PAM50 assay, but a surrogate test, based on immunostaining, would be highly desirable for routine clinical use. Briefly, proposed surrogate immunohistochemical definitions for basal-like breast cancer typically rely on lack of expression of ER, PR and HER2. To improve specificity of this so-called **triple negative** definition, positively expressed basal-like biomarkers, such as CK5, CK5/6 and EGFR, are used by some labs. In addition to self-assessment, immunopanel performance was also assessed after independent review of all ER, PR, HER2 and basal-like biomarker slides returned to cIQc. Re-assessments were performed by personnel at the Genetic Pathology Evaluation Centre (GPEC) in Vancouver, British Columbia.

With more sensitive ER testing in current use, an underlying concern that motivated this run was that there would be widespread ER positivity in basal-like carcinomas; this is of potential clinical significance as basal-like carcinomas are responsive to chemotherapy, but not tamoxifen, and ER positivity in these cases could result in inappropriate treatments being used.

RESULTS

Immunopanels: Of 51 participating labs, self-assessments were reported by 50 labs. Four labs defined basal-like breast cancer based only on lack of expression of ER, PR and HER2, while remaining labs opted to incorporate at least 1 positive basal-like biomarker into their immunopanel (**Table 1**). 47 of 50 participating labs returned all slides to cIQc for independent review.

Table 1. Summary of self-assessed immunostains used to define basal-like breast cancer cases in addition to ER, PR and HER2 at participating laboratories.

Additional Immunostains Included	No. of labs
None	4
EGFR and CK5	2
EGFR and CK5/6	1
CK5 and CK14	1
p63 and CK5/6	1
CK5	13
CK5/6	27
CK5/14	1
TOTAL	**50**

In total, the tissue microarray contained 15 basal-like cases, 23 non-basal-like cases and 2 uninterpretable cases. When possible, basal-like breast cancer was immunohistochemically-defined in three different ways:

- "*Triple Negative Basal-like*" = ER/PR/HER2 negative
- "*Core Basal-like*" = ER/PR/HER2 negative and basal-like marker(s) positive (Cheang et al., 2008)
- "*Core Basal-like (no PR)*" = ER/HER2 negative and basal-like marker(s) positive. (Nielsen et al., 2004)

Based on self-assessed results, sensitivity for detection of basal-like breast carcinomas ranged from 13-100%, while specificity ranged from 76-100% (left half of **Table 2**). Similarly, evaluation from independent review revealed sensitivities ranging from 6.7-100% and specificities ranging from 73-100% (right half of **Table 2**).

ER: In general, ER immunohistochemical analysis in this run was satisfactory. However, the higher proportion of ER-negative cases selected for inclusion in the tissue microarray appears to have led to more false positive staining in Labs 103, 106, 113, 114, 123, 135, 138, 139, 155, 164, 188, 191, and 198 *based on self-assessments*. While slides from Labs 106, 134 and 200 were not available for re-assessment, ER slides from all other participating labs were subjected to independent review. Major discrepancies between self-assessment and independent review were noted in Labs 103, 113, 114, 123, 135, 139, 164, 191, 198, 199, and 201 (**Figure 1**), with all but one lab (Lab 199) self-reporting more ER positive cores than independent review. Based on independent review, a significant issue with false positives ER staining, compared to other labs, was only evident in Labs 138, 188 and 199.

Self-assessment from Lab 170 reported the highest number of ER false negative results compared to other labs, but independent review determined that the number of ER negative cores was within the expected range. However, both self-assessment and independent review of ER staining from Lab 201 indicated a higher number of false negatives, with both nuclear ER staining and counterstaining observed to be quite faint, while there was considerable cytoplasmic background staining in some cores. Similarly, cytoplasmic staining was noted in Labs 124, 155, and 156, while membranous staining was noted in Lab 189; however, this background staining did not interfere with appropriate assessment of nuclear ER staining. As expected, false positive ER staining reported by independent review greatly influenced all immunopanels for detection of basal-like breast cancer as illustrated in **Table 2**.

PR: Once again due (presumably) to the generally higher proportion of PR-negative cases included in the tissue microarray, greater inconsistency in PR staining was detected across participating labs compared to previous cIQc runs (**Figure 2**). For some labs, this variability greatly influenced the *Core basal-like* definition (ER/PR/HER2 negative and basal-like biomarker(s) positive) for identification of basal-like breast cancer, which was particularly problematic in Labs 111, 125, 139, 155, 157, 186, and 191. Exclusion of PR from the immunopanel (i.e. *Core basal-like (no PR)* definition) led to a significant improvement in immunopanel performance for some labs experiencing PR staining issues. Notably, drastically sub-optimal performance of the triple negative definition (ER/PR/HER2 negative) for Lab 153 could be entirely attributed to the unexpectedly high number of false positive PR results reported by self-assessment and confirmed by independent review.

Generally higher PR positivity rates were observed in Labs 102, 112, 115, 119, 125, 138, 139, 150, 152, 155, 164, 170, 186, 189, 191, and 198 based on self-assessment. Although slides from Labs 106, 134 and 200 were not available for re-assessment, PR slides from all other participating labs were independently reviewed. Major discrepancies between self-assessment and independent review were noted in Labs 125, 153, 157, 164, 170, 186, and 191 (**Figure 2**). Specifically, three labs (125, 153 and 157) self-reported more PR negative cores than independent review, while the remaining discordant labs (164, 170, 186 and 191) self-reported more PR positive cores than independent review. Based on independent review, generally higher PR positivity rates were confirmed in Labs 102, 112, 115, 119, 125, 150, 152, 153, 155, 186, 189, 191 and 198; these PR positive cases were ER negative, at least in some instances, supporting the interpretation that there was false positive PR staining. Labs 111 and 157 were observed to have higher PR positivity rates by independent review but not self-assessment. With regards to general observations of PR staining noted during independent review, considerable cytoplasmic and/or stromal background staining was observed in Labs 111, 115, 124, 152, 153, 157, 190 and 201.

HER2: Both self-assessment and independent review of HER2 slides were relatively consistent across participating labs. Interobserver variability was evident, with independent review reporting a generally higher number of 3+ HER2 cores that were previously self-reported as 2+ (**Figure 3**). Overall concordance with FISH results was also very good by both self-assessment and independent review.

To the best of our knowledge, the association between HER2 immunohistochemistry and HER2-enriched PAM50 molecular subtype assignment has not yet been surveyed in clinical labs. Run 27 was not designed for this this purpose and, at this time, cIQc cannot comment on such results until further studies are performed with a larger sample size.

Basal-like biomarkers: Although Garrattograms for basal-like biomarkers (**Figure 4**) have been merged to include assessment of CK5 (16 labs in total), CK5/6 (29 labs in total), EGFR (Labs 101, 114 and 119), CK14 (Lab 107 and 124) and p63 (Lab 109), unexpectedly high variation was observed for overall staining and interpretation of basal-like biomarkers in participating labs. Since labs were asked to stain and score basal-like biomarkers according to established protocols at each facility, the following discussion focuses on the results of independent review, which strictly interpreted lack of tumour cell staining as negative and presence of any staining in tumour cells as positive. Please note that CK5, CK5/6, CK14 and EGFR were evaluated for cytoplasmic and/or membranous staining, while p63 was evaluated for nuclear staining only.

With the exception of slides from Labs 106 and 200, which were not available for re-assessment, independent review of basal-like biomarker slides was performed for all participating labs. A majority of false positive cores reported by independent review could be attributed to CK5/6 cytoplasmic staining in tumour cells. Labs using CK5 were observed to have significantly fewer false positive cores than labs that had used CK5/6. Due to the large variety of antibody clones and protocols employed for staining and interpretation of basal-like biomarkers, specific sources of variation cannot be identified at this time. Nevertheless, the significant discordance between self-assessment and independent review that is evident in several labs suggests that both standards for staining and interpretation must be established prior to potential implementation of immunopanels including positive markers for specific identification of basal-like breast carcinomas.

RUN 27 CONCLUDING COMMENTS

Using a tissue microarray enriched for basal-like breast carcinoma cases, unexpectedly higher variation in ER and PR immunohistochemical analysis was detected in cIQc Run 27. Identification of basal-like breast cancer in clinical labs was further complicated by very considerable variation in the staining and interpretation of positive basal-like biomarkers, indicating that ***standardization efforts are necessary before any attempts to implement basal-like immunopanels in a clinical setting can be made***. For your information, all reported staining protocols in each lab can be found in Supplementary Tables 1-4 at the end of this document.

A further specific concern that we wished to examine was whether a significant percentage of labs were seeing ER and/or PR positive basal-like breast cancers. The triple negative phenotype (negative for ER, PR and HER2) is the most widely used surrogate for molecular testing in the diagnosis of basal-like breast cancer, and has been used as an entrance criterion for clinical trials of novel therapies for this aggressive breast cancer subtype. A higher incidence of false positive ER staining was observed in three labs and was possibly linked to a specific combination of antibody clone and detection system as used in the two most problematic labs, who also happened to be the only labs to report use of such a protocol. As cIQc continues to work closely with these labs to address this apparent ER staining issue, cIQc Run 27 has provided a clear demonstration of the advantages of participating in immunohistochemistry external quality assurance programs. Further studies are required to specifically comment on the many sources of PR variation, but it is noteworthy that some of the false positive PR cases were ER negative, and such a combination of results should be viewed with skepticism in practice. Your regular participation in cIQc is greatly appreciated, and we look forward to working with you and the Canadian Association of Pathologists – Association canadienne des pathologistes in the future as we continue to improve our external quality assurance services. We apologize for the delay in issuing this report, but considerably more internal analysis was required, as a result of full re-assessment of all slides, which led to this delay.

REFERENCES

Cheang, Maggie CU, et al. "Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype." *Clinical Cancer Research* 14.5 (2008): 1368-1376.

Nielsen, Torsten O., et al. "Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma." *Clinical Cancer Research* 10.16 (2004): 5367-5374.

Table 2. Sensitivity, specificity and kappa agreement values comparing PAM50-defined basal-like cases versus immunohistochemically-defined "basal-like" cases.

	Based on Clinical Laboratory Self-assessments												Based on GPEC Independent Review											
	Triple Negative				Core Basal-like				Core Basal-like (no PR)				Triple Negative				Core Basal-like				Core Basal-like (no PR)			
	Sens (%)	Spec (%)	n	Kappa	Sens (%)	Spec (%)	n	Kappa	Sens (%)	Spec (%)	n	Kappa	Sens (%)	Spec (%)	n	Kappa	Sens (%)	Spec (%)	n	Kappa	Sens (%)	Spec (%)	n	Kappa
Lab 101	93.3	86.4	37	0.780	93.3	86.4	37	0.780	93.3	86.4	37	0.780	93.3	82.6	38	0.734	93.3	87.0	38	0.785	93.3	87.0	38	0.785
Lab 102	73.3	87.0	38	0.610	60.0	95.7	38	0.591	66.7	95.7	38	0.653	66.7	87.0	38	0.549	60.0	95.7	38	0.591	66.7	95.7	38	0.653
Lab 103	60.0	87.0	38	0.486	46.7	100.0	38	0.514	53.3	100.0	38	0.580	73.3	87.0	38	0.610	53.3	95.7	38	0.526	66.7	95.7	38	0.653
Lab 105	86.7	86.4	37	0.723	73.3	95.5	37	0.710	73.3	95.5	37	0.710	80.0	86.4	37	0.664	66.7	95.5	37	0.649	73.3	95.5	37	0.710
Lab 106	66.7	91.3	38	0.601	53.3	95.7	38	0.526	60.0	95.7	38	0.591												
Lab 107	80.0	86.4	37	0.664	60.0	95.5	37	0.586	66.7	95.5	37	0.649	80.0	91.3	38	0.721	66.7	95.7	38	0.653	73.3	95.7	38	0.715
Lab 109	86.7	82.6	38	0.677	66.7	95.7	38	0.653	66.7	95.7	38	0.653	86.7	87.0	38	0.728	80.0	95.7	38	0.774	80.0	95.7	38	0.774
Lab 111	73.3	86.4	37	0.603	46.7	95.5	37	0.455	60.0	95.5	37	0.586	60.0	86.4	37	0.479	40.0	95.5	37	0.387	66.7	95.5	37	0.649
Lab 112	66.7	87.0	38	0.549	40.0	100.0	38	0.447	53.3	95.7	38	0.526	53.3	91.3	38	0.474	46.7	100.0	38	0.514	60.0	95.7	38	0.591
Lab 113	73.3	87.0	38	0.610	46.7	95.7	38	0.461	46.7	95.7	38	0.461	73.3	91.3	38	0.662	53.3	95.7	38	0.526	53.3	95.7	38	0.526
Lab 114	73.3	91.3	38	0.662	73.3	91.3	38	0.662	73.3	91.3	38	0.662	86.7	87.0	38	0.728	86.7	87.0	38	0.728	86.7	87.0	38	0.728
Lab 115	66.7	91.3	38	0.601	46.7	100.0	38	0.514	66.7	95.7	38	0.653	66.7	91.3	38	0.601	66.7	100.0	38	0.708	80.0	95.7	38	0.774
Lab 119	73.3	82.6	38	0.559	73.3	87.0	38	0.610	86.7	87.0	38	0.728	73.3	86.4	37	0.603	73.3	90.9	37	0.656	86.7	90.9	37	0.776
Lab 123	73.3	87.0	38	0.610	60.0	95.7	38	0.591	60.0	95.7	38	0.591	86.7	87.0	38	0.728	66.7	95.7	38	0.653	66.7	95.7	38	0.653
Lab 124	66.7	82.6	38	0.499	53.3	95.7	38	0.526	73.3	95.7	38	0.715	66.7	86.4	37	0.542	66.7	95.5	37	0.649	80.0	95.5	37	0.771
Lab 125	60.0	95.7	38	0.591	26.7	100.0	38	0.306	46.7	95.7	38	0.461	46.7	95.7	38	0.461	33.3	100.0	38	0.377	73.3	95.7	38	0.715
Lab 126	93.3	87.0	38	0.785	80.0	91.3	38	0.721	80.0	91.3	38	0.721	93.3	86.4	37	0.780	86.7	90.9	37	0.776	86.7	90.9	37	0.776
Lab 127	80.0	82.6	38	0.619	60.0	95.7	38	0.591	66.7	95.7	38	0.653	73.3	91.3	38	0.662	60.0	100.0	38	0.645	73.3	95.7	38	0.715
Lab 129	73.3	82.6	38	0.559	53.3	95.7	38	0.526	73.3	95.7	38	0.715	60.0	95.5	37	0.586	46.7	95.5	37	0.455	60.0	95.5	37	0.586
Lab 134	80.0	81.8	37	0.612	60.0	95.5	37	0.586	66.7	95.5	37	0.649												
Lab 135	60.0	91.3	38	0.538	40.0	95.7	38	0.393	40.0	95.7	38	0.393	73.3	95.5	37	0.710	73.3	95.5	37	0.710	80.0	95.5	37	0.771
Lab 138	66.7	91.3	38	0.601	40.0	100.0	38	0.447	40.0	100.0	38	0.447	80.0	100.0	38	0.829	66.7	100.0	38	0.708	66.7	100.0	38	0.708
Lab 139	60.0	82.6	38	0.436	33.3	95.7	38	0.324	53.3	95.7	38	0.526	73.3	78.3	38	0.510	60.0	91.3	38	0.538	80.0	91.3	38	0.721
Lab 145	93.3	82.6	38	0.734	66.7	95.7	38	0.653	66.7	95.7	38	0.653	100.0	78.3	38	0.740	86.7	95.7	38	0.833	86.7	95.7	38	0.833
Lab 147	80.0	87.0	38	0.670	60.0	95.7	38	0.591	66.7	95.7	38	0.653	73.3	90.9	37	0.656	66.7	95.5	37	0.649	80.0	95.7	38	0.774
Lab 150*	80.0	87.0	38	0.670									73.3	86.4	37	0.603								
Lab 151	93.3	78.3	38	0.684	73.3	91.3	38	0.662	73.3	91.3	38	0.662	80.0	91.3	38	0.721	66.7	95.7	38	0.653	66.7	95.7	38	0.653
Lab 152	66.7	82.6	38	0.499	46.7	95.7	38	0.461	60.0	95.7	38	0.591	60.0	81.8	37	0.427	60.0	81.8	37	0.427	73.3	82.6	38	0.559
Lab 153*	33.3	90.9	37	0.265									6.7	95.7	38	0.027								
Lab 155	33.3	87.0	38	0.221	26.7	100.0	38	0.306	50.0	95.7	37	0.498	33.3	91.3	38	0.272	33.3	100.0	38	0.377	60.0	95.7	38	0.591
Lab 156	93.3	81.8	37	0.728	80.0	95.5	37	0.771	80.0	95.5	37	0.771	80.0	81.8	37	0.612	73.3	81.8	37	0.552	73.3	82.6	38	0.559
Lab 157	93.3	77.3	37	0.677	40.0	95.5	37	0.387	40.0	95.5	37	0.387	40.0	95.5	37	0.387	33.3	95.5	37	0.318	66.7	90.9	37	0.595
Lab 160	73.3	87.0	38	0.610	40.0	95.7	38	0.393	40.0	95.7	38	0.393	80.0	85.7	36	0.657	66.7	95.2	36	0.644	66.7	95.2	36	0.644
Lab 161	86.7	86.4	37	0.723	33.3	95.5	37	0.318	33.3	95.5	37	0.318	86.7	81.0	36	0.664	66.7	95.2	36	0.644	66.7	95.2	36	0.644
Lab 164	73.3	82.6	38	0.559	53.3	91.3	38	0.474	66.7	91.3	38	0.601	86.7	87.0	38	0.728	66.7	95.7	38	0.653	66.7	95.7	38	0.653
Lab 167	93.3	76.2	36	0.670	33.3	95.2	36	0.312	33.3	95.2	36	0.312	93.3	81.8	37	0.728	80.0	90.9	37	0.717	80.0	90.9	37	0.717
Lab 170	73.3	78.3	38	0.510	53.3	91.3	38	0.474	60.0	91.3	38	0.538	86.7	81.0	36	0.664	66.7	90.5	36	0.588	73.3	90.5	36	0.650
Lab 179	100.0	82.6	38	0.789	60.0	91.3	38	0.538	60.0	91.3	38	0.538	100.0	82.6	38	0.789	73.3	91.3	38	0.662	73.3	91.3	38	0.662
Lab 186	40.0	87.0	38	0.290	26.7	91.3	38	0.201	66.7	91.3	38	0.601	53.3	90.9	37	0.467	33.3	95.5	37	0.318	60.0	95.5	37	0.586
Lab 187	80.0	81.8	37	0.612	66.7	86.4	37	0.542	80.0	86.4	37	0.664	86.7	82.6	38	0.667	73.3	91.3	38	0.662	73.3	87.0	38	0.610
Lab 188	46.7	91.3	38	0.408	40.0	95.7	38	0.393	40.0	95.7	38	0.393	33.3	91.3	38	0.272	26.7	95.7	38	0.252	26.7	95.7	38	0.252
Lab 189*	60.0	100.0	38	0.645									60.0	95.7	38	0.591								
Lab 190	93.3	77.3	37	0.677	53.3	95.5	37	0.521	53.3	95.5	37	0.521	80.0	90.5	36	0.712	73.3	95.2	36	0.706	80.0	95.2	36	0.767
Lab 191	26.7	95.5	37	0.247	13.3	95.5	37	0.101	53.3	90.9	37	0.467	46.7	95.5	37	0.455	40.0	100.0	37	0.442	73.3	95.5	37	0.710
Lab 194	86.7	82.6	38	0.677	53.3	87.0	38	0.422	53.3	87.0	38	0.422	86.7	87.0	38	0.728	66.7	95.7	38	0.653	66.7	95.7	38	0.653
Lab 198*	60.0	95.7	38	0.591									60.0	95.5	37	0.586	40.0	100.0	37	0.442	53.3	95.5	37	0.521
Lab 199	73.3	82.6	38	0.559	53.3	87.0	38	0.422	53.3	87.0	38	0.422	40.0	90.5	36	0.327	20.0	95.2	36	0.170	20.0	95.2	36	0.170
Lab 200	40.0	95.7	38	0.393	26.7	100.0	38	0.306	26.7	100.0	38	0.306												
Lab 201	66.7	91.3	38	0.601	60.0	91.3	38	0.538	60.0	91.3	38	0.538	80.0	81.8	37	0.612	73.3	81.8	37	0.552	73.3	72.7	37	0.451
Lab 202	73.3	95.5	37	0.601	53.3	95.5	37	0.521	66.7	95.5	37	0.649	86.7	86.4	37	0.723	86.7	90.9	37	0.776	93.3	90.9	37	0.834

*Laboratory provided self-assessments for ER, PR and HER2 only.

Triple Negative = ER/PR/HER2 negative; **Core Basal-like** = ER/PR/HER2 negative and basal-like biomarker(s) positive; **Core Basal-like (no PR)** = ER/HER2 negative and basal-like biomarker(s) positive

Kappa Interpretation: 0.01-0.20 = Slight agreement, 0.21-0.40 Fair agreement, 0.41-0.60 = Moderate agreement, 0.61-0.80 = Substantial agreement, 0.81-0.99 = Almost perfect agreement. Immunopanels with less than moderate agreement (kappa <0.41) are indicated by red text.

Figure 1. ER Garrattogram from self-assessment and independent review.

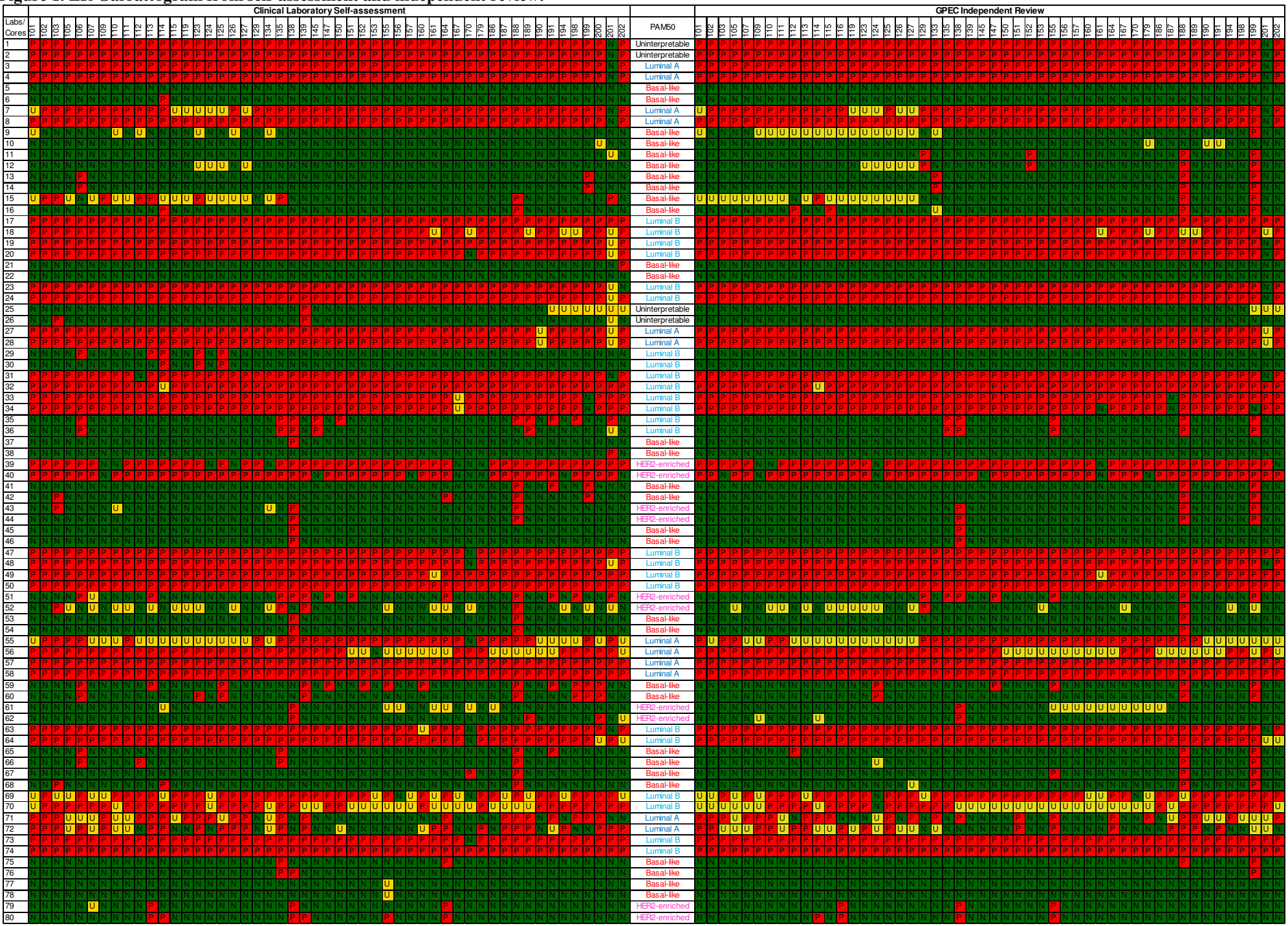

Figure 2. PR Garrattogram from self-assessment and independent review.

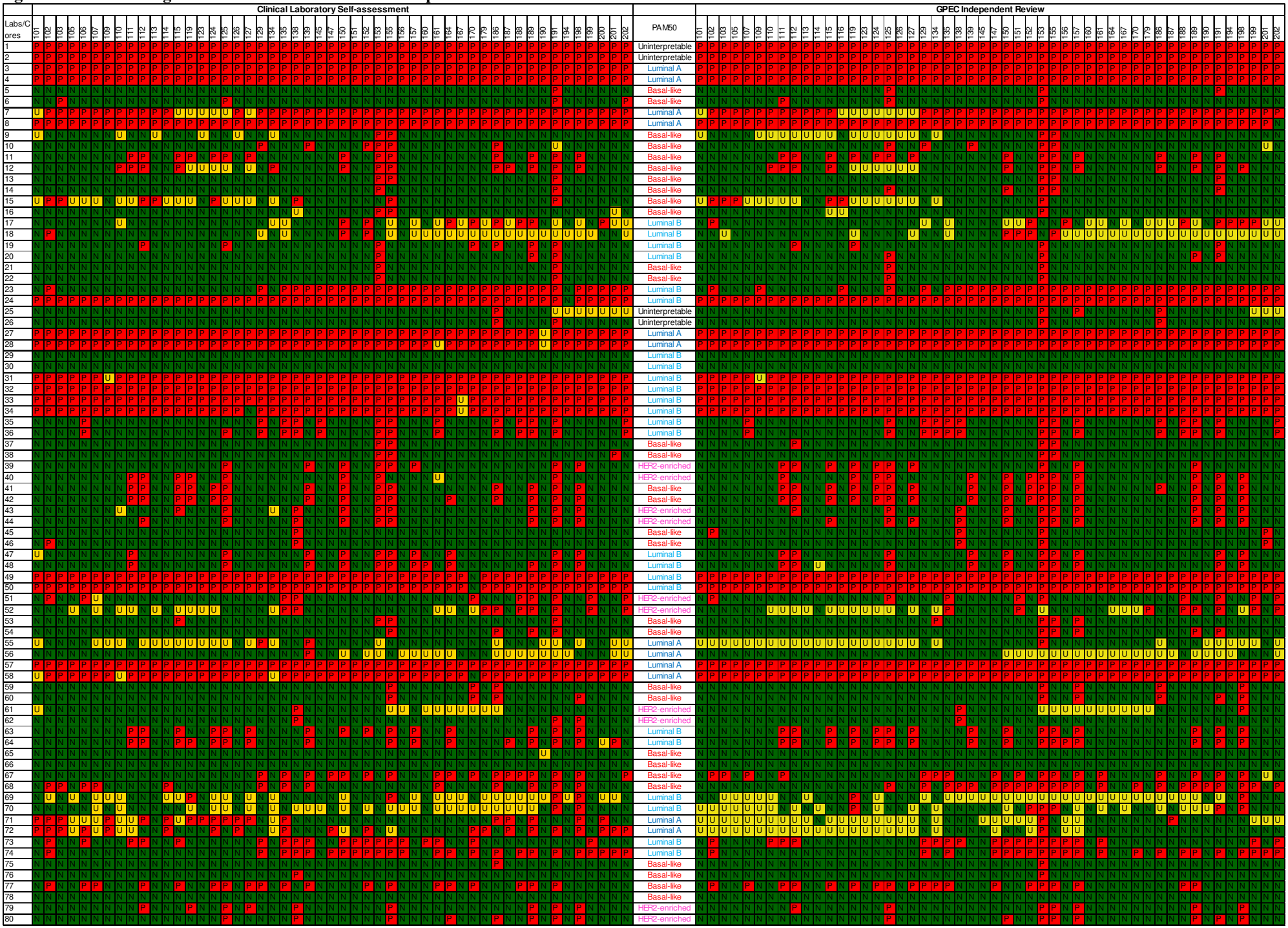

Figure 3. HER2 Garrattogram from self-assessment and independent review.

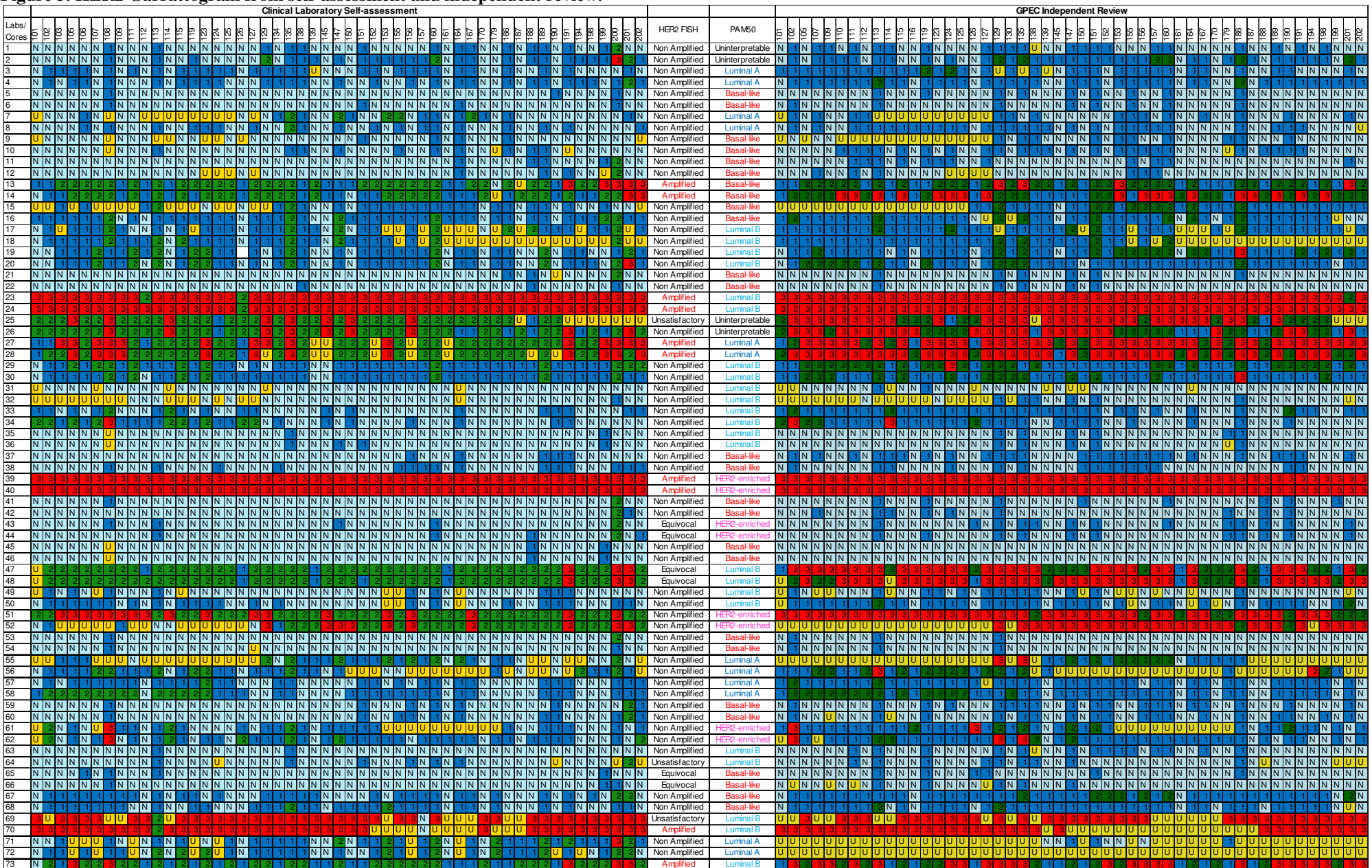

Figure 4. Basal-like biomarker Garrattogram from self-assessment and independent review.

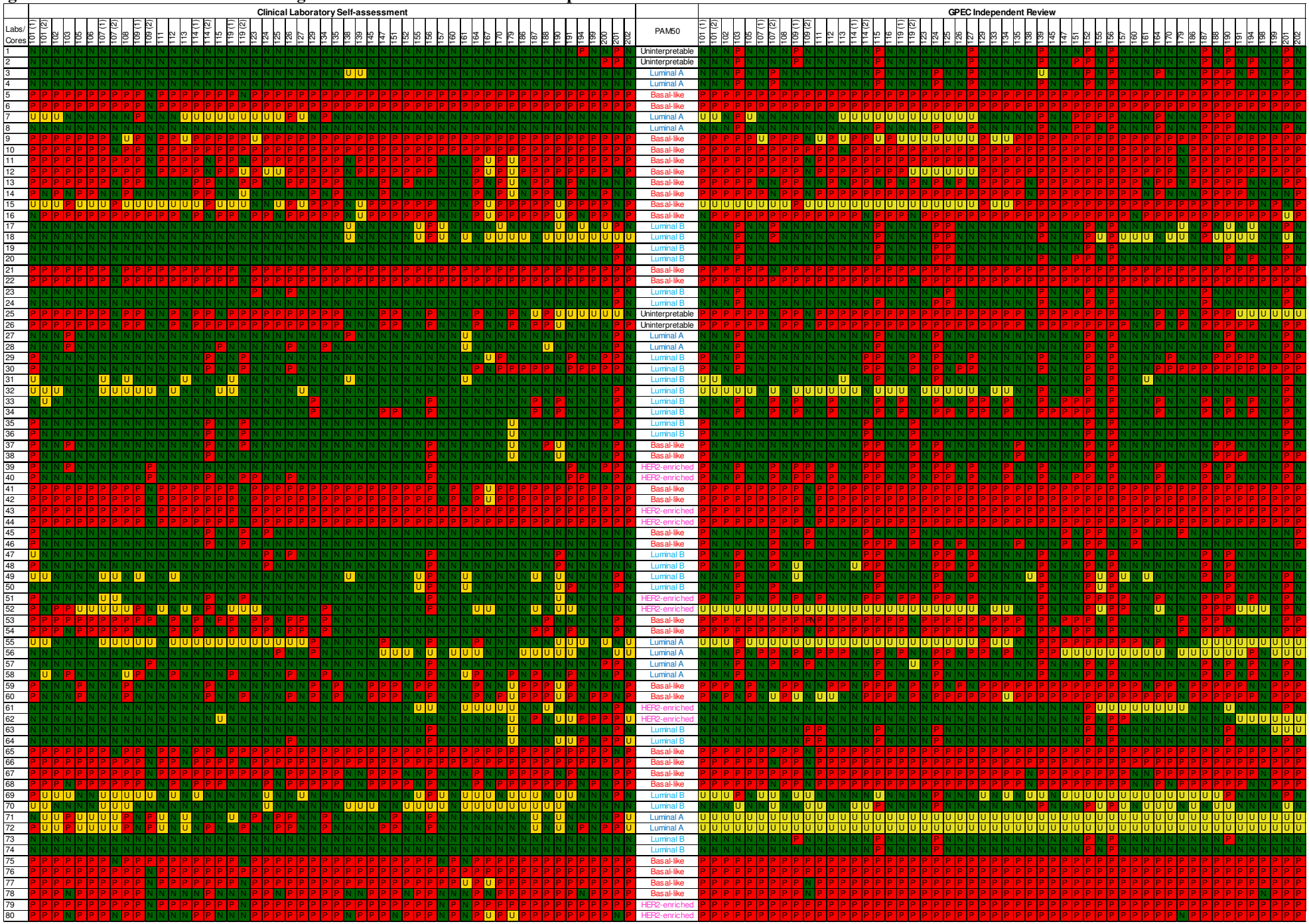

Table S1. Reported ER staining protocols.

Lab	Clone	Dilution	Vendor	Antigen Retrieval	Detection System	Amplification	Chromagen
101	SP1	1:50	NeoMarkers	CC1	Opti-view	no	DAB
102	SP1	1/30	Labvision	Dako FLEX High TRS	Envision+	None	DAB+
103	SP1	pre-diluted	Ventana/Roche	CC1 STD	IVIEW DAB	NONE	DAB
105	SP1	1:50	Thermo Scientific	CC1 Standard	Ultramap anti-Rb HRP	None	DAB
106	6F11	1:75	Vector	115C microwave 3 min	Elite (b/a)	none	DAB
107	SP1	pre-diluted	Ventana	Ultra cc1 36 min	ultraView	no	DAB
109	6F11	1/40	Vector	CC1 STD	ULTRAVIEW	NONE	DAB
110	SP1	pre-diluted	Ventana	CC1	Ultraview	N/A	DAB
111	SP1	pre-diluted	Ventana	CC1 36 MINUTES	ULTRAVIEW	NO	DAB
112	SP1	RTU	Ventana	CC1	ultraVIEW	no	DAB
113	SP1	pre-diluted	Ventana/Roche	CC1	Ultraview	n/a	DAB
114	SP1	1/50	Thermo Fisher	CC1-32min	OptiView Ventana	oView Copper	DAB
115	SP1	pre-diluted	Ventana	CC1	I-VIEW BAB	COOPER	DAB
119	SP1	pre-diluted	Ventana	HIER/CC1	Ultraview	-	DAB
123	SP1	pre-diluted	Ventana/Roche	CC1 mild	ultraview	no	DAB
124	SP1	1/100	Cell Marque	HIER	Streptavidine	CCI	DAB
125	SP1	pre-diluted	Roche	ER2-20	Leica Bond Refine detection	N/A	DAB
126	SP1	1:200	Lab Vision/Thermo	(Manual) Pressure cooker pH6	Dako Envision	None	
127	SP1	pre-diluted	Ventana	yes	Ventana UltraView Kit	no	DAB
129	Sp1	1:50	Thermo Fisher	Bond ER2 High pH Retrieval Buffer	Bond Refine Detection Kit	No	DAB
134	SP1	pre-diluted	Ventana	CC1	Ventana Ultraview	N/A	DAB
135	SP1	1:50	Thermo Fisher	20 min Buffer PH 9	Bond Refine	No	DAB
138	EP1	RTU	Dako	High pH	Envision Flex HRP	none	DAB
139	SP1	RTU	Ventana	CC1, 30 min.	Iview DAB	no	DAB
145	SP1	1/10 of pre-diluted	Ventana	CC1 30 mins	XT ULTRAVIEW DAB v3	YES VENTANA	DAB
147	SP1	1:50	Thermo Fisher	(ER2)EDTA, pH9 20min	Bond Polymer Refine	N/A	DAB
150	sp1	RTU	Ventana	cc1	ultraview	copper	DAB
151	SP1	1:50	Thermo Fisher	pH9.0/20 MIN	BOND VISION	N/A	DAB
152	SP1	pre-diluted	Ventana/Roche	CC1 mild	Iview	not applicable	DAB
153	SP1	RTU	Ventana	CC1	Ultraview	Copper	DAB
155	SP1	pre-diluted	Ventana	CC1	Ultraview	Iview copper	DAB
156	SP1	RTU	Ventana	CC1	I-View	none	DAB
157	SP1	pre-diluted	Ventana	CC1 30 MIN.	BENCHMARK XT	YES	YES
160	SP1	RTU	Ventana	EDTA pH8	Ventana Ultraview	no	DAB
161	EP1	RTU	Dako	High EDTA TRIS	Envision FLEX	no	DAB
164	SP1	pre-diluted	Ventana	CC1 / 36 min.	Benchmark Ultra	no	DAB
167	SP1	pre-diluted	Ventana	Cell conditionner #1	XT ultraView v3	none	DAB
170	Ep1	RTU	Dako	yes	Envision flex (polymer)	no	DAB
179	6F11	1:200	Leica/nonocastra	Citrato pH6,0	Novolink, Leica	Novolink, Leica	DAB
186	SP1	1:100	Thermoscientific	HIER EDTA buffer 20'	Bond Refine Detection	None	DAB
187	SP1	pre-diluted	Ventana	CC1	Optiview	None	DAB
188	6F11	1/40	Leica	ER1(20)	Bond Refine	None	DAB
189	SP1	pre-diluted	Ventana	CC1	UltraView DAB	none	Copper
190	SP1	pre-diluted	Ventana	cc1-30 min	ventana	none	DAB
191	SP1	RTU	Ventana/Roche	cc1	ultraview	none	DAB
194	SP1	pre-diluted	Ventana	CC1	AVIDIN/BIOTIN	AMP.KIT VENTANA A/B	DAB
198	SP1	Pre-diluted - 24 min	Roche/Ventana	CC1 mild	Ultraview		DAB
199	6F11	RTU	Leica	HIER 20 mins	Bond Refine	no	yes
200	SP1	RTU	Cell Marque	CC1S	ultraView	N/A	DAB
201	ER EP1	1:100	Epitomics	Citrate, pH6	AVIDIN/BIOTIN	None	DAB
202	6F11	1/100	Vector	CITRATE Ph6.0 10 MIN AT 123c	MACH 4 POLYMER- BIOCARE	none	DAB

Table S2. Reported PR staining protocols.

Lab	Clone	Dilution	Vendor	Antigen Retrieval	Detection System	Amplification	Chromogen
101	16	1:100	Novocastra	CC1	Opti-view	no	DAB
102	16	1/125	Leica	Dako FLEX High TRS	Envision+	None	DAB+
103	100	pre-diluted	Roche/Ventana	CC1 MILD	ULTRAVIEW	NONE	DAB
105	PgR636	1:50	Dako	CC1 standard	Ultramap anti-Ms HRP	None	DAB
106	PgR1294	1:1250	Dako	115C microw ave 3 min	Elite (b/a)	none	DAB
107	PgR 1294	1:50	Dako	Ultra cc1 64 min	ultraView	yes	DAB
109	16	1/100	Vector	CC1 STD	ULTRAVIEW	NONE	DAB
110	100	pre-diluted	Ventana	CC1	Ultraview	N/A	DAB
111	100	pre-diluted	Ventana	CC1 36 MINUTES	ULTRAVIEW	NO	DAB
112	100	RTU	Ventana	CC1	ultraVIEW	no	DAB
113	PgR 636	1/300	DAKO	Low pH PT link	Flex	+Mouse	DAB
114	16	1/25	Novocastra	CC1-32min	OptiView Ventana	oView Copper	DAB
115	100	pre-diluted	Ventana	CC1	I-VIEW DAB	COOPER	DAB
119	16	pre-diluted	Ventana	HIER/CC1	Ultraview	-	DAB
123	16	1/50	Vector	CC1 standard	ultraview	no	DAB
124	IE2	none	Ventana	HIER	Streptavidine	CCI	DAB
125	100	1/4 of pre-diluted	Roche	ER2-20	Leica Bond Polymer Refine detection	N/A	DAB
126	Pgr636	1:500	Dako	Pressure cooker pH6	Small Polymer	none	
127	100	pre-diluted	Ventana	yes	Ventana ultraView Detection Kit	no	DAB
129	16	1:400	Novocastra	Bond ER2 High pH Retrieval Buffer	Bond Refine Detection Kit	No	DAB
134	IE2	pre-diluted	Ventana	CC1	Ventana Ultraview	N/A	DAB
135	16	1:400	Leica	20min Buffer PH 9	Polymer DAB	No	DAB
138	636	RTU	Dako	High pH	Envision Flex HRP	none	DAB
139	100	RTU	Ventana	CC1, 30 min.	Iview DAB	no	DAB
145	100	1/4 of pre-diluted	Ventana	CC1 30 mins	XT ULTRAVIEW DAB v3	YES VENTANA	DAB
147	16	1:800	NCL	(ER2)EDTA,pH9,20mins	Bond Polymer Refine	N/A	DAB
150	100	RTU	Ventana	cc1	ultraview	copper	DAB
151	1A6	1:200	NCL	pH6.0/20 MIN	BOND VISION	N/A	DAB
152	1E2 (250)	pre-diluted	Roche/Ventana	CC1 mild	Iview	not applicable	DAB
153	x	x	x	x	x	x	x
155	100	pre-diluted	Ventana	CC1	Ultraview	Iview copper	DAB
156	x	x	x	x	x	x	x
157	IE 2	pre-diluted	Ventana	CC1 30 MIN.	BENCHMARK XT	YES	YES
160	100	no	Ventana	EDTA pH8	Ventana Ultraview	no	DAB
161	PgR636	RTU	DAKO	High EDTA buffer TRIS	Envision FLEX	mouse linker	DAB
164	100	pre-diluted	Ventana	CC1 / 36 min.	Benchmark Ultra	no	DAB
167	100	pre-diluted	Ventana	Cell conditionner #1	XT ultraView v3	none	DAB
170	PgR636	RTU	dako	yes	Envision flex (polymer)	no	DAB
179	16	1:400	Leica/Novocastra	citrato pH6,0	Novolink,leica	Novolink,leica	DAB
186	PR88	1:100	Biogenex	HIER EDTA buffer 20'	Bond refine detection	None	DAB
187	IE2	pre-diluted	Ventana	CC1	Ultraview	None	DAB
188	PR(16)	RTU	Leica	ER2(20)	Bond Refine	None	DAB
189	100	pre-diluted	Ventana	CC1	UltraView DAB	none	Copper
190	16	1:50	Novocastra	CCI 30min	Ventana	none	DAB
191	100	RTU	Roche	CC1	Ultraview	none	DAB
194	100	pre-diluted	Ventana	CC1	AVIDIN/BIOTIN	NONE	DAB
198	100	Predilute- 20min	Roche/Ventana	CC1 mild	Ultraview		DAB
199	PGR16	1:50	Leica/Novocastra	HIER 20mins	DAB	no	yes
200	Y85	RTU	Cell Marque	CC1S	ultraView	N/A	DAB
201	PR - EP2	not provided	Epitomics	not provided	not provided	not provided	not provided
202	1.60E 01	RTU	Leica	CITRATE Ph9.5 30 MIN	LEICA REFINE DETECTION KIT	none	DAB

Table S3. Reported HER2 staining protocols.

Lab	Clone	Dilution	Vendor	Antigen Retrieval	Detection System	Amplification	Chromogen
101	SP3	1:50	NeoMarkers	CC1	Opti-view	no	DAB
102	SP3	1/450	Labvision	Dako FLEX High TRS	Envision+	None	DAB+
103	4B5	pre-diluted	Roche/Ventana	CC1 MILD	ULTRAVIEW	NONE	DAB
105	4B5	Neat	Ventana	CC! standard	DABMAP	heat	DAB
106	4B5	pre-diluted	Ventana	90C, 36 min	Ultraview	none	DAB
107	4B5	pre-diluted	Ventana	Ultra cc1 36 min	UltraView	no	DAB
108	SP3	1:200	Thermo Fisher	pH6 pressure cooker 6 min @125C	Dako Rabbit Envision	none	Tyramide-Cy5
109	4B5	RTU	Ventana	CC1 MILD	ULTRAVIEW	NONE	DAB
111	4B5	pre-diluted	Ventana	CC1 36 MINUTES	ULTRAVIEW	NO	DAB
112	4B5	RTU	Ventana	CC1	ultraView	no	DAB
113	Polyclonal	1/150	Dako	CC1	Ultraview	n/a	DAB
114	SP3	1/200	Thermo Fisher	CC1-32min	OptiView Ventana	oView Copper	DAB
115	4B5	pre-diluted	Ventana	CC1	I-VIEW DAB	COOPER	DAB
119	A0485	1/1000	Dako	HIER/CC1	Ultraview	-	DAB
123	4B5	pre-diluted	Roche/Ventana	CC1 mild	ultraview	no	DAB
124	4B5	none	Ventana	HIER	Streptavidine	CCI	DAB
125	4B5	pre-diluted	Roche	CC1-mild	ultraView Universal DAB	N/A	DAB
126	SP3	1:450	Lab Vision	HIER pH10	Small Polymer	None	
127	4B5	pre-diluted	Ventana	yes	Ventana ultraView Detection Kit	no	DAB
129	A0485	1:600	Dako	Bond ER 1 Low pH Retrieval Buffer	Bond Refine Detection Kit	No	DAB
134	4B5	pre-diluted	Ventana	CC1 30 minutes	Ventana Ultraview	N/A	DAB
135	A0485	1:700	Dako	20 min Buffer PH6	Polymer DAB	NO	DAB
138	-	1:600	Dako	Low pH	Envision Flex HRP	none	DAB
139	4B5	RTU	Ventana	CC1, 30 min.	Eview DAB	no	DAB
145	SP3	1/600	Cell Marque	CC1 30 mins	XT ULTRAVIEW DAB v3	YES VENTANA	DAB
147	Polyclonal	1:500	Dako	(ER1)Citrate,pH6,20min	Bond Ploymer Refine	N/A	DAB
150	sp3	1/100	NeoMarkers	cc1	ultraview	copper	DAB
151	MDA-175	1;350	Dako	pH6.0	BOND VISION	N/A	DAB
152	4B5	pre-diluted	Roche/Ventana	CC1 standard	Iview	not applicable	DAB
153	x	x	x	x	x	x	x
155	4B5	pre-diluted	Ventana	CC1	Ultraview	Iview copper	DAB
156	x	x	x	x	x	x	x
157	4B 5	pre-diluted	Ventana	CC1 60 MIN.	BENCHMARK XT	YES	YES
160	A0485	1/700	Dako	EDTA pH8	Ventana Ultraview	no	DAB
161	HER2	RTU	Dako	Herceptest retrieval solution	Herceptest visualisation reagent	no	DAB
164	4B5	pre-diluted	Ventana	CC1 / 36 min.	Benchmark Ultra	no	DAB
167	A0485	1/1700	Dako	Cell conditionner #1	XT ultraView v3	none	DAB
170	ErB-2	RTU	Dako	yes	herceptest dako	no	DAB
179	CB11	1;250	Leica	citrate pH6,0	Novolink, Leica	Novolink, Leica	DAB
186	Polyclonal	1:200	Dako	HIER Citrate buffer 20'	Bond Refine Detection	None	DAB
187	4B5	pre-diluted	Ventana	CC1	Optiview	None	DAB
188	CB11	RTU	Leica	ER1(25)	Oracle HER2 IHC System	None	DAB
189	4B5	pre-diluted	Ventana	CC1	ultraView DAB	n/a	Copper
190	SP3 rabbit	1:50	Thermo Scientific	CCI 30 min	Ventana	none	DAB
191	4B5	RTU	Roche/Ventana	CC2	ultraview	none	DAB
194	4B5	pre-diluted	Ventana	CC1	AVIDIN/BIOTIN	CC1	DAB
198	4B5	predilute -16 min	Roche/Ventana	CC1 mild	Ultraview		DAB
199	CB11	RTU	Leica	HIER 25 mins	DAB	no	yes
200	CB11	RTU	Cell Marque	CC2S	ultraView	N/A	DAB
201	HER2 EP3	not provided…	Epitomics	not provided...	not provided..	not provided.	not provided
202	HER2	RTU	Dako	EPITOPE RETREIVAL SOLUTION	HERCEPTEST VISUALIZATION KIT	none	DAB

Table S4. Reported basal-like biomarker staining protocols.

Lab	Clone	Dilution	Vendor	Antigen Retrieval	Detection System	Amplification	Chromagen
101 (1)	EGFR						
101 (2)	CK5						
102	CK5	1/100	Leica	Dako FLEX High TRS	Envision+	None	DAB+
103	CK5/6	pre-diluted	Ventana/Roche	CC1 52MINS	ULTRA VIEW	NONE	DAB
105	CK5	1:50	Novacastra	CC1 Standard	DABMAP	none	DAB
106	CK5	1:100	Leica/Novocastra	100C microw ave 5 min	MACH 4 mouse	none	DAB
107 (1)	CK5/6	1:50	Dako	cc1 standard	ultraView	no	DAB
107 (2)	CK14	1:50	ID Labs	cc1 standard	ultraView	yes	DAB
108	CK5	1:200	Novacastra	pH9 pressure cooker 3min @125C	Dako Mouse Envision Kit	none	
109 (1)	CK5/6	RTU	Ventana	CC1 STD	ULTRAVIEW	NONE	DAB
109 (2)	p63	RTU	Ventana	CC1 STD	ULTRAVIEW	NONE	DAB
111	CK5/6	1/200	Dako	CC1 64 MINUTES	ULTRAVIEW	NO	DAB
112	CK5/6	RTU	Ventana	CC1	optiView	no	DAB
113	CK5/6	1/100	Dako	High pH PT link	Flex	+Mouse	DAB
114 (1)	CK5	1/100	Novocastra	CC1-32min	OptiView Ventana	oView Copper	DAB
114 (2)	EGFR	1/20	Invitrogen/Zymed	P1-4min	OptiView Ventana	oView Copper	DAB
115	CK5/6	1:20	NeoMarker	CC1	I-View DAB	Cooper	DAB
119 (1)	CK5/6	1/50	Dako	HIER/CC1	Ultraview	-	DAB
119 (2)	EGFR	pre-diluted	Ventana	Enzyme protease 1	Ultraview	-	DAB
123	CK5	1/100	Leica	CC1 standard	ultraview	no	DAB
124	CK5/14	RTU	Cell Marque	HIER	Streptavidine	CCI	DAB
125	CK5/6	1/400	Dako	ER2-20	Leica Bond Polymer Refine	N/A	DAB
126	CK5	1:100	Leica	HIER pH6	Small Polymer	None	
127	CK5/6	RTU	Ventana	antigen retrieval	UltraView		DAB
129	CK5/6	1:150	Dako	Bond ER2 High pH Retrieval Buffer	Bond Refine Detection Kit	No	DAB
134	CK5/6	1:100	Dako	DAKO Ph9	Dako Ension Mouse plus	N/A	DAB
135	CK5	pre-diluted	Leica	20 min Buffer PH 6	Polymer DAB	No	DAB
138	CK5/6	RTU	Dako	High pH	Envision Flex HRP	none	DAB
139	CK5/6	RTU	Ventana	CC1, 60 min.	Iview DAB	no	DAB
145	CK5	1/50	Cell Marque	CC1 30 mins	XT ULTRAVIEW DAB v3	YES VENTANA	DAB
147	CK5/6	1:150	Dako	(ER2)EDTA,pH9,20min	Bond Polymer Refine	N/A	DAB
151	CK5/6	1;100	Dako	pH9.0/20 MIN	BOND VISION	NA	DAB
152	CK5/6	pre-diluted	Ventana/Roche	CC1 standard	Iview	not applicable	DAB
155	CK5	1:200	Leica (Novocastra)	CC1	Ultraview	Iview copper	DAB
156	CK5/6	x	x	x	x	x	x
157	CK5/6	1/100	INVITROGEN	ST-AC44	BENCHMARK XT	YES	YES
160	CK5/6	1/50	Dako	EDTA pH8	Ventana Ultraview	no	DAB
161	CK5/6	RTU	Dako	High EDTA buffer TRIS	Envision Flex	no	DAB
164	CK5/6	pre-diluted	Ventana	CC1 / 52 minutes	Benchmark Ultra	none	DAB
167	CK5/6	1/50	Dako	Cell conditionner #1	XT ultraView v3	none	DAB
170	CK5/6	RTU	Dako	yes	Envison flex (polymer)	no	DAB
179	CK5	1:100	Leica	tris-Edta	Novolink, leica	Novolink, leica	DAB
186	CK5	1:100	Cell Marque	HIER in EDTA buffer	Bond Refine detection	None	DAB
187	CK5/6	pre-diluted	Ventana	CC1	Optiview	None	DAB
188	CK5	1/50	Leica Biosystems	ER2(20)	Bond Refine	None	DAB
190	CK5/6	1:50	Dako	CCI/Standard	iView Ventana	None	DAB
191	CK5	1/10	Leica (novocastra)	CC1	ultraview	none	DAB
194	CK5/6	1:10	Dako	MILD CC1 VENTANA	AVIDIN/BIOTIN VENTANA	NONE	DAB
198	CK5/6						
199	CK5/6	1:50	Cell Marque	HIER 20mins	DAB	no	yes
200	CK5/6	RTU	Cell Marque	CC1S	ultraView	N/A	DAB
201	CK5/6	not provided	Epitomics	not provided	not provided	not provided	not provided
202	CK5	RTU	Leica	CITRATE Ph9.5 20 MIN	LEICA REFINE DETECTION KIT	none	DAB