Expert Assessment for CIQC Run 29

Assessment performed in May, 2013, at Vancouver General Hospital.

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This run was an inaugural run to assess HER2 immunostaining for gastric cancer biopsies. After a clinical trial showing that targeted therapy against HER2, in patients whose gastric cancers show amplification/overexpression of HER2, there are increasing numbers of requests for laboratories to assess HER2 in either small biopsies or resection specimens. Although in some initial studies there were technical issues with IHC assessment of HER2 status in gastric cancers, it has been our experience that with modern reagents and well optimized protocols, it is relatively straight forward to successfully assess gastric samples for any laboratory that is already doing breast cancer HER2 assessment. There is a subtle difference in the criteria for assessment of HER2 immunostaining, i.e. no minimum required percentage for membranous staining and 3+ scores allowed for cases with apical loss, between breast and gastric cancers, but otherwise the experience we have gained in HER2 IHC assessment of breast cancer is relevant.

A significant difference between gastric and breast cancers is the much higher likelihood of heterogeneity of HER2 amplification in gastric cancers compared to breast cancers. We saw evidence of this as some of the cores used for this run were from samples that showed HER2 amplification elsewhere in the tumor, but were HER2 unamplified/not expressed in the cores used for this assessment. While it is important to be aware of intratumoral heterogeneity it was not assessed in this run.

The first task for the reviewers was to identify those cores where there was no tumour or insufficient tumour to allow assessment of staining. These cores were then removed from further consideration. We next did correlation of staining for each lab, compared to the reference results, based on their reported results (as recorded on the CIQC website). This allowed us to identify those labs with lesser levels of agreement with reference laboratory results (i.e. kappa statistic < 0.80) so that particular attention could be paid to those laboratories results.

Based on review of the Garratograms, HER2 staining results were highly concordant between laboratories. In fact, the six of 45 cores with HER2 amplification were identified, based on either 2+ or 3+ staining, by all but one laboratory. Only lab 161 had false negative results on these cores. There were only 2 labs (lab 164 and 200), that recorded ANY false positive results (defined as 3+ staining in a core where HER2 was not overexpressed. Put another way, 35 of 38 labs had both 100% sensitivity and 100% specificity (kappa = 1.0) for HER2 testing on gastric cancer, based on this 45 case challenge. Congratulations!

A variety of reagents were successfully used, but most labs were using either SP3 or 4B5 primary antibodies. We suspect that the excellent performance on this assessment, compared to historic results for HER2 assessment in gastric cancer, reflect this widespread use of these newer rabbit monoclonal antibodies. Because of the overall excellent results, individual lab assessments will only be done for laboratories 161, 164 and 200.

Comments on individual laboratories:

161: staining, overall, was considerably weaker than in the other laboratories, resulting in false negative results. On reviewing the protocol, we noted that 'Herceptest' reagents were used.

Although FDA approved, most labs have stopped using these, as the newer rabbit monoclonal antibodies deliver cleaner staining results (more intense with less background) in routine use, and consideration could be given to switching primary antibodies.

164: False positive staining results were recorded for cores 5 and 15. In the reviewer's opinion the staining was technically adequate, but there was interpretive error in considering these cores to show 3+ staining, as in our opinion neither met criteria for '3+ positivity', and we would have scored them as 2+ and 1+, respectively. In summary, there was technically excellent staining, but attention should be focused on correct interpretation and assignment of '3+ positivity'.

200: Multiple false positive results were recorded on self assessment. No slide was returned, at the time of the assessment meeting, and therefore the reviewers cannot comment on the technical quality of staining or interpretation.

In-situ hybridization (ISH) component

Seventeen laboratories participated in the in-situ hybridization portion of the run. This consisted of two tissues, one considered to be 'clearly amplified' and the other to be 'non-amplified' by a reference site. Although the IHC-FISH concordance rate in gastric cancer is lower than in breast cancer (83% vs. 95%, respectively) the breast cancer scoring criteria are used for gastric cancer: Her2:Cep17 ratio >2.0 defining a FISH HER2-positive tumor [Wolff AC, Hammond ME, Schwartz JN, et al: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 25:118–145, 2007].
Overall the test results were very satisfactory. Importantly, there was no appreciable difference in terms of hybrization time, probe or instrument used.
The 'amplified' test sample (sample 2) was recognized accordingly by all seventeen participants. The 'non-amplified' sample (sample 1) was tested as 'amplified' by three sites (109, 137 and 160) while two sites arrived at an 'equivocal' result (110 and 113). There was a wide variation in counting both Cep 17 and HER2 signals by all the laboratories resulting in the variation of the final results. Although, the intratumoral heterogeneity may account for some of the differences and consideration to more extensive nuclei counts may be given.

Comments on individual laboratories:

116: Could not be evaluated as no counts were provided.

113: Could not be evaluated in detail, as no Cep17 signal counts provided for Sample 2.

136: Presumed clerical error as reversed scores for HER2 and Cep 17 signals were provided.

100: The lab counted a large number of Cep 17 signals and while achieving a correct result, miscounting signals in equivocal cases could potentially lead to erroneous reporting.

IHC HER2 Laboratory Self Assessment

RUN 29 cIQc — HER2 IHC

Labs/Cores	101	103	105	108	109	110	111	113	114	115	117	119	125	126	127	136	137	138	145	149	150	152	153	156	157	160	161	162	164	167	170	175	186	189	190	191	199	200
1	0	0	1	0	0	0	0	1	U	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
2	0	2	2	0	1	1	1	1	0	2	0	0	1	1	1	0	0	1	0	2	0	2	0	2	2	2	0	2	2	1	0	1	2	2	0	2	0	2
3	1	1	2	1	1	1	1	1	0	2	0	0	1	1	1	0	0	0	0	1	0	2	0	2	2	2	U	2	2	1	0	1	1	2	1	2	0	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0	0
5	2	2	2	1	1	1	1	2	2	2	2	1	2	1	1	1	2	2	1	2	1	2	1	2	2	2	U	2	3	2	2	2	2	2	2	2	0	3
6	1	0	1	1	1	1	1	1	0	1	2	0	0	1	1	0	2	1	0	2	1	1	1	2	2	1	0	1	1	1	0	0	1	2	1	0	0	2
7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	2	3	2	3	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
8	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	U	3	3	3	U	3	3	3	3	3	3
9	0	1	U	U	0	0	1	1	U	1	0	U	1	0	0	0	0	0	0	0	0	1	0	U	U	0	U	U	1	U	0	U	0	2	0	U	0	U
10	0	0	U	1	0	0	0	0	0	0	0	U	0	U	0	0	0	0	0	0	U	0	0	0	0	0	0	0	U	0	0	0	0	U	0	0	0	1
11	0	0	1	0	0	0	0	0	0	0	0	0	1	0	U	0	1	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1
12	0	1	1	0	1	1	1	1	0	0	0	0	1	1	1	0	0	1	0	2	0	2	0	2	2	2	1	2	1	1	0	1	1	1	0	1	0	1
13	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1	1	2	1	1	1	1	1	0	2	1	0	2	1	1	0	2	1	0	2	1	2	1	2	2	2	0	2	2	1	1	2	1	2	2	2	0	2
15	0	2	2	1	2	1	2	2	0	2	0	1	2	1	2	1	1	2	0	2	1	2	0	2	2	2	0	2	3	2	1	2	2	2	1	2	0	3
16	0	0	0	0	U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
17	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1
18	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	1	0	1	1	1	0	0	1	1	0	0	0	2
19	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	2	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
20	3	3	3	3	3	2	3	3	3	3	3	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3
21	0	1	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	0	2	0	1	0	2	1	0	0	1	1	1	0	0	1	0	0	1	0	2
22	0	1	1	0	1	1	0	0	0	1	0	0	1	1	0	0	0	1	0	2	0	1	0	2	1	1	0	2	1	1	1	1	1	1	0	1	0	2
23	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
24	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
25	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
29	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	0	0	2
30	0	1	0	0	0	1	2	1	0	0	0	0	2	0	1	0	0	0	0	0	0	1	0	2	1	0	1	U	1	0	2	0	1	0	0	0	0	2
31	3	3	3	2	3	3	3	3	3	3	2	2	3	2	3	2	3	3	2	3	2	3	2	3	3	3	1	3	3	3	3	3	3	3	2	3	3	3
32	3	3	3	2	2	2	3	3	3	3	3	2	3	2	3	2	3	2	2	3	2	3	2	3	3	3	1	3	3	3	3	3	3	3	3	3	2	3
33	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	1	1	0	0	0	1	1	0	0	0	1
34	2	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	2
35	1	1	2	U	1	1	1	1	0	2	0	0	2	1	1	0	0	1	0	1	0	1	0	2	2	2	0	2	2	1	1	2	2	2	0	2	0	3
36	0	1	1	0	1	0	1	1	0	1	0	0	1	1	0	0	0	0	0	2	0	1	0	2	2	2	0	1	2	1	0	1	2	2	0	1	0	3
37	0	1	0	U	0	0	1	1	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	2	1	1	0	1	1	1	0	1	1	1	0	1	0	3
38	1	0	1	0	1	1	1	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	2	1	1	0	1	1	1	1	1	1	1	1	0	0	2
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
40	0	0	U	U	U	0	0	0	U	U	0	U	0	U	0	0	0	U	U	0	U	U	0	U	0	0	0	0	U	U	0	U	1	U	U	U	0	U
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	1	1	1	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0	2	0	1	0	2	2	2	0	1	2	1	1	2	2	2	1	1	0	2
43	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	2	1	0	0	0	0	0	0	1	1	0	0	0	0	2
44	0	0	0	U	U	0	0	0	0	0	0	U	0	U	0	0	0	0	U	0	0	0	0	0	U	0	U	0	0	U	0	0	1	U	U	0	0	0
45	1	0	0	1	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	U	0	0	1	0	1	0	0	2

3	3+ staining
2	2+ staining
1	1+ staining
0	Negative
U	Unsatisfactory

IHC HER2 Laboratory Protocols

		RUN 29 cIQc				HER2 IHC						
Laboratories/Fields	101	103	105	108	109	110	111	113	114	115	116	117
Clone	SP3	4B5	4B5	SP3	4B5	4B5 Ventana Her2/neu	4B5	Polyclonal	SP3	4B5	SP3	SP3
Dilution	1:100	PRE	Neat	1:100	RTU	pre-diluted	PREDILUTE	1/175	1:200	prediluted	1/100 (lot 1210A)	1/200
Vendor	Thermo Scientific	Ventana	Ventana	Fisher Scientific	VENTANA	Ventana/Roch	VENTANA	DAKO	Thermo Fisher	Ventana	ThermoScientific	Thermo scientific
Antigen Retrieval	Heat	CC1mild	CC1 standard	pH6 PC6 (Dako TRS)	HIER-CC1MILD(30MIN)	CC1, Mild 30 minute conditioning	CC1 36 minutes	CC1	CC1-32min	CC1	CC1 court	CC1
Detection System	Ventana Optiview	Ultra View	DABMAP	Dako Rabbit Envision Kit	Ultraview universal DAB detection kit	Ventana UltraView Universal Dab	ULTRAVIEW	Ultraview	Optiview Ventana	IView DAB	ultraView DAB Ventana	Ultra-view dab
Amplification		NA	none	None	no	N/A	ONLY COPPER	n/a	oView Copper	Cooper	non	none
Chromogen	Ventana Optiview (brown)	DAB	DAB	Dako Rabbit Envision Kit	DAB	DAB	DAB	DAB	DAB	DAB	DAB	Dab

Laboratories/Fields	119	125	126	127	136	137	138	145	149	150	152	153
Clone	A0485	4B5	SP3	4B5	A0485	SP3	A0485	SP3	AO485	sp-3	4B5	SP3
Dilution	1/1000	predilute	1:450	predilute	1/600	1/150	1:600	1/600	1:500	1/100	Pre-Diluted	1/100
Vendor	Dako	Ventana	ThermoFisher	Ventana/Roche	Dako	Fisher Scientific	DAKO	CELL MARQUE	Dako	Neomarkers	Ventana	Thermo Fisher
Antigen Retrieval	CC1 30 MIN	CC1 standard	HIER pH10	yes	none	buffer,pH7.4, 36min. @ 95 Deg C	FLEX TRS Low pH	CC1 30 mins	PT Link low pH 98 C 20 min	cc1	CC1 Standard	CC1 30 min.
Detection System	Ultraview Ventana	Ventana UltraView	Envision	Ventana ultraView	LSAB	Ventana, Ultra View, polymer	Envision FLEX + /HRP	XT ULTRAVIEW DAB v3	Envision Flex	ultraview	iVIEW	Ultraview Dab
Amplification	-	N/A	none	n/a	no	none	None	YES, VENTANA	Envision Flex Rabbit Linker	copper	Copper Sulfate	Ultraview Cooper
Chromogen	DAB	DAB	DAB	DAB	DAB	DAB	DAB	DAB	DAB	dab	DAB	Dab

Laboratories/Fields	156	157	160	161	162	164	167	170	175	186	189
Clone	4B5	4B 5	4B5	RABBIT ANTI-HUMAN her2 PROTEIN	4B5	4B5	A0485	herceptest	4B5	Polyclonal Rabbit	4B5
Dilution	pre-diluted	PRE DILUTED	no	RTU	RTU	predilute	1/1700	ready to use	Predilute	1:200	Predilute
Vendor	Ventana	VENTANA	Dako	DAKO	VMS	Ventana	Dako	dako	v	Dako	Ventana
Antigen Retrieval	CCI	CC1 60 MIN.	EDTA pH8	Herceptest retrieval solution	Mild/CC1	ultra CC1	CC1	yes	EZ Prep	HIER, citrate buffer , 20'	CC1 Mild
Detection System	iview	BENCHMARK XT OPTIVIEW	Ventana Ultraview	Herceptest visualisation reagent	UltraView	BenchmarkUltra	Ultraview universal DAB	envision flex(polymer)	I-View	Bond refine detection system	UltraView DAB
Amplification	-	YES	no	no	no	no	Copper	no	I-vew	None	none
Chromogen	DAB	YES	DAB	DAB	DABv3	DAB	DAB	dab	DAB	DAB	DAB

Laboratories/Fields	190	191	199	200
Clone	SP3	4B5	CB11	CB-11
Dilution	1:50	RTU	RTU	RTU
Vendor	thermoscientific	Roche	Leica	Cell Marque
Antigen Retrieval	cc1 mild (30 minutes)	CC2 36 min	HIER 25mins	CC2S
Detection System	Ventana XT	ultravieuw	DAB	UltraView
Amplification	Amplification	none	N/A	N/A
Chromogen	IViewDAB	DAB	yes	DAB

ISH Laboratory Self Assessment and Protocols

HER2	ISH			cIQc	Run 29					HER2	ISH			
Laboratories/Fields	103	105	109	110	113	114	115	125	136	137	138	160	175	186
Sample 1 - Average # Cep 17 signals:	2.1	4.2	2.6	2.45	n/a	3.2	3.9	1.6	4.68	2.1	(40/20) 2	3.81	4.35	3.05
Sample 1 - Average # HER2 signals:	3.7	4.6	8.4	5.3	5.3	4.6	3.57	2.1	10.1	5.95	(52/20) 2.6	10.62	5.2	4.2
Result	Non Amplified	Non Amplified	Amplified	Equivocal	Equivocal	Non Amplified	Non Amplified	Non Amplified	Equivocal	Amplified	Non Amplified	Amplified	Non Amplified	Non Amplified
Sample 2 - Average # Cep17 signals:	1.2	2.4	2.1	23	n/a	2.8	2.3	1.6	23.83	1.5	(49/20) 2.45	2	2.1	2.4
Sample 2 - Average # HER2 signals:	19.5	13.3	17.8	277	>10	>10.0	13.2	6.3	2.73	12.9	(277/20) 13.85	>4	20.85	13.9
Result	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified	Amplified
Probe	Her2Dnp Chr17Dig (C probe)	LSI HER-2/neu Sp O/ CEP 17 Sp G DNA Probe	Her2 DNA, Chr 17	Her2DNP Chr17Dig	Inform Her2 DNA Probe	Her-2 DNA Probe Kit	Her2 Neu / C17	Inform HER2 DNA probe	dual LSI Her2 spectrum orange/CEP17 spectrum green	HER2DNP+CHR17DIG	HER2/CEN-17 Probe mix	Path Vysion	Inform Ventana	Pathvysion Her2 FISH
Vendor	Roche	PathVysion	Ventana	Ventana	Ventana/Roche	Vysis PathVysion	Vysis Path Vysion	Ventana	Vysis	Ventana Medical System/Roche	Dako	Abbott Molecular	Ventana	Abbott Molecular
Instrument	Benchmark Ultra	na Manual	Benchmark XT	BenchMark XT	Benchmark XT	Vysis Hybrite	Hibridizu Thermobrite	Benchmark Ultra	manual except for hybridization on hybrite	Benchmark Ultra,Ventana	Hybridizer	Thermobrite	Benchmark	Manual + incubation in Hybrite
Hybrization Time	6 hrs	16-18 hours	6 HOURS	6 hours	6 hours	16 hours	15 hours	6 hr	10 min at 73degC, overnight at 37	6 hours	20 hours	18hrs	4	16 hr
Pretreatment Time	16 mins	50min	CC2 EXT(32MIN),ISH P3(8MIN)	CC2 buffer for 48 minutes @90degrees and Protease 3 for minutes @ 37 degrees	4 min	95C/40min,RT/20 min	30 minutes	8 min with prot3	as per vysis instructions	TRIS buffer,pH 7.4-7.8 for 3 cycles totalling 28 minutes followed by protease 3 for 20 minutes	10 min	Pepsin, 20min; Thiocyanate, 15min	16 mins	20'
Post Hybridization Wash: Reagent, Temp, Time:	Silver wash 72deg 8 mins	2 X ssc/0.3% NP40 @ 72C for 2 min	SILVER WASH 11 @72-24MIN	SSC buffer @72 degrees for 24 minutes	Silver Wash II, 72C x 3 for 8 min each rinse	Buffer(20xSSC+NP40), 73C, 2min	SSC, 72C, 8min	SSC, 69 C, 8 min X3	2 min at 73 degC in SSC/NP40 buffer as per vysis instructions	Sodium Chloride Sodium Citrate buffer, 3 cycles 8 min. each at 72 Deg C	Stringent Wash @ 65C, 10 min	2xSSC, 4min, 65C	Rinse buffer, 72 degrees, 8 mins	20xSSC, 73 degree, 2.5 min