Assessors' report for cIQc Run 100: Gastric HER2 IHC

Assessors: B Gilks, D Ongaru, J Won (recorder)

OVERVIEW

Run 100 Gastric HER2 consisted of a 45-core tissue microarray of gastric carcinomas. As per previous reports, heterogeneity of HER2 amplification in gastric cancers was observed in some cores and is well documented in the literature. For the purposes of this run the small tissue microarray cores were evaluated as if "biopsy" specimens. As such, the scoring system that laboratories were asked to apply was

- **0** – no reactivity or membranous reactivity in <5* tumour cells
- **+1** – faint/barely perceptible membranous reactivity in ≥5* tumour cells
- **+2** – weak to moderate complete or basolateral membranous reactivity in ≥5* tumour cells
- **+3** – moderate to strong complete or basolateral membranous reactivity in ≥5* tumour cells
- **Unsatisfactory (U)** – technical problem that makes interpretation impossible, such as core drop off or no tumour cells present

**5 cells corresponds to a "tumor cell cluster" in the recently published guidelines for interpretation of HER2 staining based on BIOPSY specimens. Note that for resection specimens, the guidelines are different (i.e. >10% of cells) as per the same guidelines. For the purposes of this exercise we are considering the small TMA cores to be "biopsy" specimens.* (Bartley AN, Washington MK, Colasacco C et al. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. J Clin Oncol 2017; 35: 446-464.)

Testing for HER2 overamplification and overexpression in gastric carcinoma remains challenging. The heterogeneity of staining is one issue, interpretation of expression is slightly different than for breast cancer is another, and there remain issues with the correlation between protein expression and amplification, and cases with borderline amplification levels. As it stands, though, detection of increased HER2 protein on the basolateral cell membrane, by IHC, is the first step in determining whether a patient is eligible for trastuzumab therapy for their gastric carcinoma. The results from this run show that all labs consistently detected increased HER2 expression on the paired cores 7&8, 19&20, and 31&32, and most labs had optimal staining, with strong expression seen in these samples and minimal background. Two general issues were noted. Labs using the Herceptest reagents were more likely to see non-specific staining e.g. staining in core 29, which does not have HER2 amplification. We have commented on the increased non-specific staining with this antibody in the past, and it has also been noted by NordiQC on some of their runs. This is not to say that one cannot get optimal staining with Herceptest. It is certainly possible, but it is apparent that labs using the rabbit monoclonal antibodies have, on the whole, better signal-to-noise ratios. Another observation in this run, as in the past, was increased background/non-specific staining that was observed with the use of avidin-biotin based detection systems. This technology was the norm in the past but it has been largely supplanted by polymer-based detection systems and we would urge labs to consider switching to the polymer-based detection systems. In summary, results from this run were excellent, with almost no false positive or negative results. There are cases where FISH (performed on whole sections) showed amplification but there was little or no evidence of protein overexpression, based on staining of the tissue microarray slides from labs across Canada. There are a number of possible explanations for this and it's important that labs not expect to see perfect correlation between IHC and FISH in these samples.

RESULTS

Participating laboratories demonstrated either optimal or adequate staining. Participant-specific feedback is provided below:

Lab ID	IHC Status	Comments
101	Adequate	Slightly weak staining
109	Optimal	
114	Adequate	Slightly weak staining
136	Adequate	False-positive staining in Cores 29 linked to HercepTest
149	Optimal	
175	Optimal	
181	Optimal	

Lab ID	IHC Status	Comments
186	Optimal	
190	Adequate	Slight background (non-specific staining) due to iView detection system
191	Optimal	
202	Adequate	False-positive staining in Cores 29 linked to HercepTest
220	Adequate	Slightly weak staining
230	Optimal	
234	Adequate	Slightly weak staining

*based on assessor consensus

Garrattogram after CIQC assessment:

Lab/ Core	101	109	114	136	149	175	181	186	190	191	202	220	230	234	FISH
1	0	0	0	0	0	0	U	0	0	0	0	0	0	0	Neg
2	0	0	0	0	1	1	0	1	1	0	0	0	0	0	Neg
3	0	0	0	0	2	0	0	1	2	1	0	1	0	0	Equiv
4	0	0	0	0	2	0	0	0	1	0	0	1	0	0	Equiv
5	0	0	0	1	2	1	0	2	2	1	2	2	1	1	Amplified
6	0	0	0	0	0	0	0	0	2	0	0	2	0	1	Amplified
7	2	3	2	3	3	3	3	3	3	2	3	3	3	3	Amplified
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
9	U	U	0	0	U	U	0	0	0	1	U	U	1	0	Neg
10	U	0	U	0	0	U	0	U	0	0	U	U	U	0	Equiv
11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Equiv
12	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Amplified
13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Neg
14	0	1	0	0	2	1	0	1	1	1	1	1	2	0	Equiv
15	0	1	0	0	2	2	1	1	2	1	0	1	2	0	Equiv
16	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Neg
17	U	U	U	U	0	U	0	U	U	0	U	U	U	U	Neg
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
19	3	3	3	3	3	3	3	3	3	3	3	3	3	2	Amplified
20	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
21	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Equiv
22	0	1	0	0	2	1	0	1	1	1	0	1	0	0	Equiv
23	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Neg
24	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Neg
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
26	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Neg
27	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Neg
28	U	0	U	U	0	0	0	U	1	0	U	U	U	U	Neg
29	0	0	0	2	0	0	0	0	2	0	3	0	U	0	Neg
30	U	U	U	U	0	0	0	0	U	0	U	U	U	0	Neg
31	2	3	2	2	3	3	3	3	3	2	3	3	3	2	Amplified
32	2	3	2	3	3	3	3	3	3	3	3	3	3	2	Amplified
33	0	0	0	0	0	0	0	1	1	0	0	0	0	0	Equiv
34	0	0	0	0	2	0	0	0	2	0	0	1	0	0	Equiv
35	0	0	0	0	2	0	0	1	1	1	0	1	1	0	Neg
36	0	0	0	0	2	1	0	0	1	2	0	1	1	0	Neg
37	U	U	U	U	0	U	0	U	U	0	U	U	0	U	Neg
38	0	0	0	2	0	0	0	0	0	0	1	0	0	0	Amplified
39	0	0	0	2	0	0	0	0	0	0	2	0	0	0	Amplified
40	U	U	U	U	0	U	U	U	U	0	U	U	U	U	Equiv
41	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Equiv
42	0	0	0	0	0	0	0	0	1	0	0	1	0	0	Neg
43	0	1	0	0	0	0	0	0	1	0	0	0	0	0	Neg
44	U	U	U	U	0	U	U	U	U	0	U	U	U	U	Neg
45	0	0	0	0	0	0	0	0	1	0	0	1	0	0	Neg

Supplementary Table 1 summarizes staining protocols, Supplementary Table 2 summarizes descriptive statistics and Supplementary Table 3 provides the definitions of CIQC IHC Statuses assigned to each participant. 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 Gastric 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	OMNIS on board high pH	30	4B5	1:8	Roche	Y21121	15	OMNIS Envision Flex	n	n	DAB
109	HIER HIGH pH CC1	36 MIN	4B5	RTU	ROCHE	E27815	16 MIN	ULTRAVIEW	N	Y	DAB
114	Envision Flex TRS,High pH	30	4B5	RTU	Roche	E27815	15	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
136	DAKO HERCEP TEST BUFFER	40	A0485	RTU	DAKO	20065068	30	DAKO HERCEP TEST	N	N	HT DAB
149	high pH OMNIS	20 min at 97 C	A0485	1:600	Dako/Agilent	20061928	20	EnVision Flex OMNIS	no	no	DAB
175	Hier	36 min	4B5	predilute	Roche	E30207	16 min	Polymer (ultraDAB)	N	Y - copper	DAB
181	CC1 on board	30 minutes	4B5	pre-diluted	Ventana/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
190	CC1	36	SP3	1:50	Thermo	9103S1701C1	40	IVIEW	Y	N	DAB
191	CC1	36	4B5	RTU	Roche	G07935	12	ultraview	N	N	DAB
202	ER buffer from HERCEPT test kit	40	AO485	Ready to use	Agilent	20061666	30	HERCEPT test kit	N	N	DAB
220	HIER	36 minutes	sp3	1:150	Thermo scientific	9103S1305H	28 minutes	ventana ultraview	N	Y	DAB
230	HIER	36	4B5	PREDILUTE	Ventana	E30207	16	ULTRAVIEW	N	N	DAB
234	Envision FLEX TRS High	30	SP3	80	Thermoscientific	9103S1701C1	17	Envision FLEX	No	No	DAB

Table S2. Descriptive statistics for Gastric HER2 IHC based on CIQC assessment. Cores 5, 6, 12, 38 and 39 were excluded from analysis.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	40	80	32	32/32 (100%)	1	1	1
109	40	85	34	34/34 (100%)	1	1	1
114	40	82.5	33	33/33 (100%)	1	1	1
136	40	85	34	34/34 (100%)	1	1	1
149	40	97.5	39	39/39 (100%)	1	1	1
175	40	85	34	34/34 (100%)	1	1	1
181	40	92.5	37	37/37 (100%)	1	1	1
186	40	85	34	34/34 (100%)	1	1	1
190	40	87.5	35	35/35 (100%)	1	1	1
191	40	100	40	40/40 (100%)	1	1	1
202	40	80	32	32/32 (100%)	1	1	1
220	40	80	32	32/32 (100%)	1	1	1
230	40	82.5	33	33/33 (100%)	1	1	1
234	40	87.5	35	35/35 (100%)	1	1	1

Table S3. IHC Status definitions.

IHC Status	Definition	CIQC 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 CIQC for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory’s accreditation provider.