Summary Report – Run 114 gastric HER2 IHC

Assessors: X Wei, J Garratt, J Won

Overview

Over-expression of HER2 in gastric cancers occurs in 15% to 25% of gastric carcinomas, and the addition of trastuzumab to conventional treatment has been shown to improve survival outcomes in HER2-overexpressing gastric cancers. As a result, HER2 testing and trastuzumab treatment are becoming the standard of care for metastatic gastric cancer. The survey consisted of 45 tissue cores of gastric carcinomas. 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 correspond to a "tumor cell cluster" in the 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. (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.)*

Results

The technical quality of staining for participants was very good. No false-positive or false-negative staining was observed. Participant-specific feedback is below:

Lab	Status	Comments
101	Optimal	
111	Optimal	
112	--	Slide not available at the time of assessment
114	Optimal	
136	Adequate	Artifactual staining linked to HercepTest
149	Optimal	Minor artifactual staining with use of Clone A0485
175	Optimal	
181	--	Slide not available at the time of assessment
186	Optimal	Slight background
190	Optimal	
202	Adequate	Artifactual staining linked to HercepTest
207	Optimal	
220	Adequate	Slightly weak staining; moderate background in some cores
230	Optimal	
234	Adequate	Artifactual staining linked to HercepTest

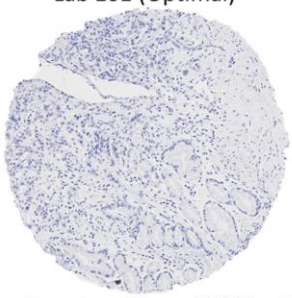
*based on CPQA assessor consensus

Garrattogram after CPQA assessment:

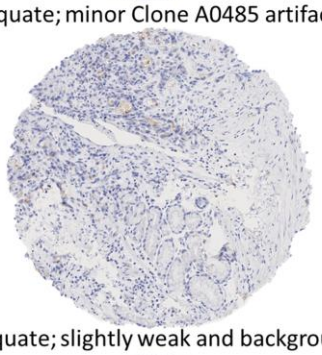
Lab/ Core	101	111	112	114	136	149	175	181	186	190	202	207	220	230	234	FISH
1	U	N	N	U	N	N	U	N	N	U	U	U	U	U	U	Neg
2	N	1	1	N	N	N	N	1	1	N	N	1	N	1	N	Neg
3	N	1	2	N	N	2	1	1	1	N	N	1	1	N	1	Equiv
4	N	N	2	N	N	1	N	N	N	N	N	N	1	N	1	Equiv
5	N	N	2	N	1	1	N	N	2	N	1	N	1	1	2	Amplified
6	N	N	1	N	N	N	N	N	N	N	N	N	1	N	N	Amplified
7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
9	U	2	U	N	N	N	U	2	U	N	N	2	U	2	N	Neg
10	N	N	U	U	U	U	U	N	U	N	N	N	U	U	N	Equiv
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Equiv
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Equiv
13	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Neg
14	N	2	1	N	N	2	1	2	1	1	N	2	1	2	1	Equiv
15	N	2	2	N	N	2	1	2	1	1	N	1	N	1	N	Equiv
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Neg
17	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Neg
18	N	N	1	U	N	N	U	N	N	N	N	N	N	N	N	Neg
19	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
20	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
21	N	N	1	N	N	N	N	N	N	N	N	N	N	N	N	Equiv
22	N	2	2	N	1	2	1	1	1	N	N	1	1	2	1	Equiv
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Neg
24	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	Neg
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Neg
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Neg
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Neg
28	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Neg
29	N	N	N	N	N	1	N	N	N	N	2	N	N	N	N	Neg
30	N	N	U	U	N	N	N	N	N	N	U	U	U	U	N	Neg
31	3	3	3	2	2	3	3	3	3	3	2	3	2	3	3	Amplified
32	3	3	3	2	3	3	3	3	3	3	2	3	2	3	3	Amplified
33	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	Equiv
34	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	Equiv
35	N	1	2	N	1	1	1	1	2	N	N	2	1	1	2	Neg
36	U	U	1	N	N	U	N	U	U	U	U	U	1	N	U	Neg
37	U	U	U	U	U	U	U	N	U	U	U	N	U	U	U	Neg
38	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Amplified
39	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	Amplified
40	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Equiv
41	N	N	N	N	N	N	N	N	N	N	N	N	1	N	N	Equiv
42	N	N	1	N	N	N	N	N	1	N	N	N	1	N	N	Neg
43	N	N	1	N	N	N	N	N	1	N	N	N	N	N	N	Neg
44	U	U	U	U	U	U	U	N	U	U	U	U	U	N	U	Neg
45	N	N	1	N	N	N	N	N	N	N	N	N	1	N	N	Neg

Figure 1. Representative images of the qualitative variability of gastric HER2 IHC.

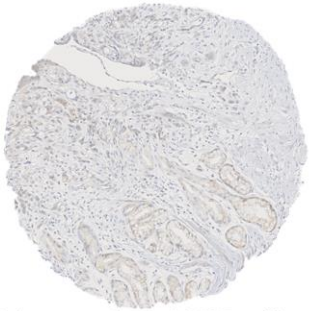
Lab 101 (Optimal)



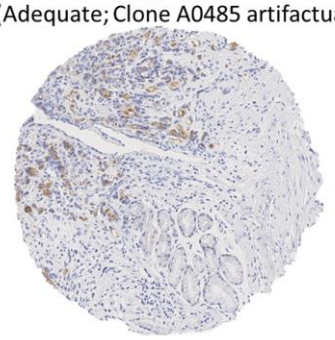
Lab 149 (Adequate; minor Clone A0485 artifactual staining)



Lab 220 (Adequate; slightly weak and background staining)



Lab 234 (Adequate; Clone A0485 artifactual staining)



Supplementary Table 1summarizes the reported staining protocols for gastric HER2 IHC, which can be referred to during validation or optimization of a staining protocol. Supplementary Table 2 summarizes descriptive statistics based on CPQA assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 3 provides the definitions of IHC Status and recommended participant action. Your regular participation in CPQA is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association canadienne des pathologistes.

This report has been updated with scanned images that were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.

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Table S1. Reported gastric HER2 staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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)
101	DAKO OMNIS	LDT	EnVision FLEX HIGH pH	30 MIN	4B5	1:8	ROCHE DIAGNOSTICS	F05675	15 MIN	EnVision FLEX	N	N
111	Benchmark Ultra	Commercial	HIER	36	4B5	predilute	ventana	F30653	32	ULTRAVIEW	N	Y
112	BOND III	LDT	BOND ER2 pH 9.0	20 minutes	4B5	1:4 ratio of RTU	Ventana/Roche	F24837	15 minutes @ RT	BOND Polymer Refine Detection	no	no
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	4B5	1:8	Roche	G01326	25	Envision FLEX DAKO Omnis	N	N
136	DAKO AS480	HERCEP TEST KIT	DAKO PT LOW	40	A0485	RTU	DAKO/AGILENT	20076620	30	DAKO ENVISION FLEX +	N	N
149	Dako OMNIS	LDT	high pH OMNIS	20 min at 97C	A0485	1:800	Dako Agilent	20076104	20	EnVision Flex OMNIS	No	No
175	Ventana	lab developed	HIER	36	4B5	predilute	Roche	g00616	16	ultra DAB	N	Y
181	Ventana Benchmark	commercial	CC1 on board	30 min.	Her2 4B5	predilute	Ventana	F30654	16 min.	Ventana Ultraview DAB	N	Y
186	LEICA BOND III	LDT	HIER	20	HER2	1:400	DAKO	20067287	15	LEICA BOND REFINE DETECTION KIT	N	N
190	Benchmark Ultra	LDT	CC1	36	4B5	predilute	Ventana	F30653	24	iView	N	N
202	DAKO/Agilent	commercial	HEIR	40	AO485	RTU	DAKO/Agilent	20075497	30	HErceptest kit	N	N
207	BenchMark Ultra	LDT	CC1-online	36	4B5	predilute	Ventana	F29872	16 minutes	Ultraview DAB	N	Y
220	BENCHMARK ULTRA	COMMERCIAL ASSAY	HIER	36	SP3	1:150	THERMO SCIENTIFIC	9103S1305H	28	VENTANA ULTRAVIEW	N	Y
230	Benchmark Ultra	LTD	HIER	32	4B5	RTU	ROCHE	F29872	16	ULTRAVIEW	N	N
234	OMNIS	LDT	PTmodule/LOW	30	POLY	200	DAKO/Agilent	20079950	20	Envision Flex	N	N

Table S2. Descriptive statistics based on CPQA assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	45	82.22	37	37/37 (100%)	1	1	1
111	45	86.67	39	39/39 (100%)	1	1	1
112	45	82.22	37	37/37 (100%)	1	1	1
114	45	80	36	36/36 (100%)	1	1	1
136	45	86.67	39	39/39 (100%)	1	1	1
149	45	84.44	38	38/38 (100%)	1	1	1
175	45	80	36	36/36 (100%)	1	1	1
181	45	91.11	41	41/41 (100%)	1	1	1
186	45	82.22	37	37/37 (100%)	1	1	1
190	45	84.44	38	38/38 (100%)	1	1	1
202	45	82.22	37	37/37 (100%)	1	1	1
207	45	84.44	38	38/38 (100%)	1	1	1
220	45	80	36	36/36 (100%)	1	1	1
230	45	84.44	38	38/38 (100%)	1	1	1
234	45	84.44	38	38/38 (100%)	1	1	1

Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining).	**PASS**
Adequate	All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation.	**PASS**
Sub-optimal	None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation.	**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]**
Unsatisfactory	Technical issue (e.g. unsuitable antibody selection, etc.)	**N/A**

1 – A one-time sub-optimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".

1,2 – Please contact the CPQA for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.