

Assessors' report for Run 109: gastric HER2

Assessors: B Gilks, J Won (recorder)

Assessment performed on May 6, 2020, at Vancouver General Hospital, British Columbia, Canada

Overview

Immunohistochemical detection of increased HER2 protein on the basolateral cell membrane of tumour cells is the first step in determining whether a patient is eligible for trastuzumab therapy as treatment for gastric carcinoma. Challenges encountered in testing for HER2 overamplification and overexpression in gastric carcinoma include heterogeneity of staining, interpretation of expression patterns that differ from those seen in breast cancer (which most pathologists are more familiar with), cases with borderline amplification levels, and issues with the correlation between protein expression and amplification.

Results

Technical quality of IHC staining for gastric HER2 was excellent for participating labs. Labs using the HercepTest reagents were more likely to see non-specific staining. This increased non-specific staining using this antibody has been observed in previous proficiency testing challenges, both with CPQA and other external proficiency testing programs. While optimal staining with HercepTest is still possible, it appears that rabbit monoclonal antibodies have generally better signal-to-noise ratios. Some tissue microarray cores had little or no evidence of protein overexpression despite FISH (performed on whole sections) showing amplification. In these instances, there may be several possible explanations e.g. sampling of areas with no overexpression/amplification for the microarray, and it's important that participants not expect to see perfect correlation between IHC and FISH in these samples.

Participant-specific feedback for gastric HER2 IHC is summarized below:

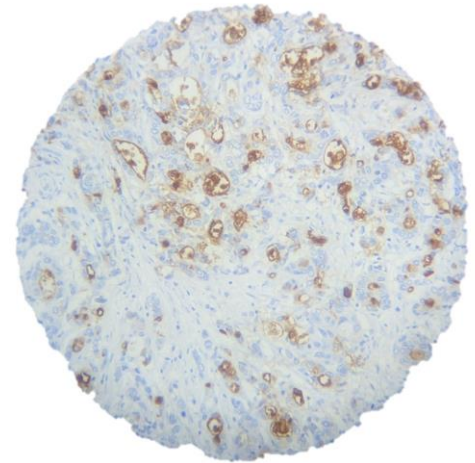
Lab ID	IHC Status*	Comment
101	Optimal	
109	Optimal	
114	Optimal	
120	--	Slide not available for assessment
136	Adequate	Artifactual staining of the luminal aspects of cells that may be attributed to use of the HercepTest
149	Optimal	
175	Optimal	
181	Optimal	
186	Optimal	
190	Optimal	
202	Adequate	Artifactual staining of the luminal aspects of cells that may be attributed to use of the HercepTest; reagents were not applied to some or only partial cores in Columns 11 and 12 of the TMA
220	Optimal	
230	Optimal	
234	Adequate	Generally weak staining

*based on assessor consensus

Garratogram after CPQA assessment of gastric HER2 IHC:

Lab/ Core	101	109	114	120	136	149	175	181	186	190	202	220	230	234	FISH
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	Neg
3	0	0	0	1	0	1	0	0	1	1	0	0	0	0	Equiv
4	0	0	0	1	0	1	0	0	1	1	1	1	0	0	Equiv
5	0	0	0	1	0	1	0	0	1	0	1	1	0	0	Amplified
6	0	0	0	0	0	0	0	0	0	1	0	1	0	0	Amplified
7	3	3	3	3	3	3	3	3	3	3	3	3	3	2	Amplified
8	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Amplified
9	0	0	0	U	0	0	1	1	1	0	0	U	0	0	Neg
10	U	U	U	U	0	U	0	0	0	0	U	U	U	0	Equiv
11	0	0	0	0	0	0	0	0	0	0	U	0	0	0	Equiv
12	0	0	0	0	0	0	0	0	0	0	U	0	0	0	Equiv
13	0	0	0	0	0	0	0	0	0	0	U	0	0	0	Neg
14	0	1	0	1	0	1	0	1	1	0	U	0	1	0	Equiv
15	0	1	0	1	0	1	1	1	1	0	0	0	1	0	Equiv
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
17	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Neg
18	0	0	0	0	U	0	0	0	0	0	0	0	U	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	0	0	0	0	0	0	Equiv
22	0	0	0	1	0	1	0	1	1	1	0	0	0	0	Equiv
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
24	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	Neg
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
28	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Neg
29	0	0	0	0	2	0	0	0	0	0	2	0	0	0	Neg
30	0	0	U	U	0	0	0	0	0	U	2	0	0	0	Neg
31	2	2	2	3	2	3	3	3	3	2	2	2	2	1	Amplified
32	3	3	3	3	2	3	3	3	3	3	2	3	3	2	Amplified
33	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Equiv
34	0	0	0	0	0	0	0	0	0	2	0	1	0	0	Equiv
35	0	1	0	1	0	1	1	1	1	0	0	0	0	0	Neg
36	0	1	0	0	0	U	0	1	1	0	U	0	0	0	Neg
37	U	U	U	U	U	U	0	0	U	0	U	U	U	U	Neg
38	0	0	0	0	0	0	0	0	0	0	2	0	0	0	Amplified
39	0	0	0	0	2	0	0	0	0	0	U	0	0	0	Amplified
40	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Equiv
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Equiv
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg
44	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Neg
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Neg

Lab 136
(Adequate)



Lab 149
(Optimal)

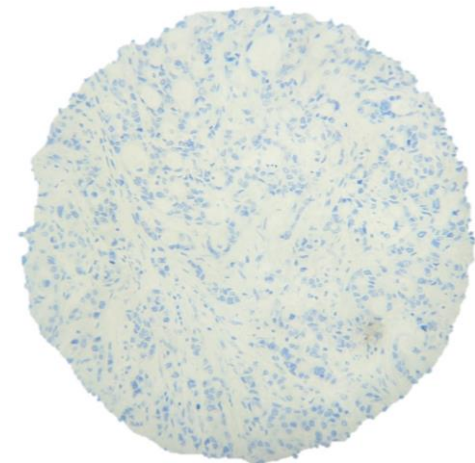


Figure 1. Representative image of the artifactual staining in the luminal aspect of cells that may be attributed to the HercepTest.

Supplementary Table 1 summarizing reported staining protocol details and Supplementary Table 2 containing 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 caution. 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 working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes for external quality assurance services.

Table S1. Reported gastric HER2 IHC 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	EnV FlexTRS, High PH	30 min	4B5	1:8	ROCHE DIAGNOSTICS	F05675	15 min	DAKO Envision Flex	N	N	DAB
114	Envision Flex TRS, High pH	30	4B5	1:8	roche	F15240	25	Envision FLEX DAKO Omnis	N	N	Envision Flex DAB
120	HIER	32 MINS	4B5	RTU	VENTANA	F20661	16 MINS	ULTRAVIEW	N	N	DAB
136	Dako Hercep Test buffer	30	A0485	RTU	Dako/ Agilent	20072143	30	Dako Hercep Test Kit	N	N	HT DAB
149	high pH OMNIS	20 min at 97 C	A0485	1:800	Dako Agilent	20062529	20	EnVision Flex OMNIS	No	No	DAB
175	HIER	36 min	4B5	pre-dilute	Roche	F20902	16 min	ULTRA DAB (polymer)	N	Y (copper)	DAB
181	CC1 on board	30 minutes	Ventana Her2neu 4B5	pre-diluted	Roche/Ventana	F02957	16 minutes	Ventana Ultraview DAB	NO	Yes	DAB
186	HIER	20	c-erbB-2	1:400	Dako	20067287	15	BOND POLYMER REFINE DETECTION	N	N	DAB
190	CC1	32	SP3	1:50	Thermo	9103S1711B	32	Optiview	N	N	DAB
202	HIER PH6.0	40	ERBB2	RTU	DAKO	20072143	30	HERCEPTEST FOR AUTOMATED LINK PLATFORMS VISUALIZATION	N	N	DAB
220	HIER	36 min	CERB-2 (SP3)	1/150	THERMO SCIENTIFIC	9103S1305H	28	VENTANA ULTRAVIEW	N	Y	DAB
230	HIER	32	4B5	predilute	Roche	F20661	16	Ultraview	N	N	DAB
234	Omnis / High	30	SP3	RTU	Cell Marque	73509	20	Envision Flex	N	N	DAB

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	41	85.37	35	35/35 (100%)	1	1	1
109	41	85.37	35	35/35 (100%)	1	1	1
114	41	82.93	34	34/34 (100%)	1	1	1
120	41	80.49	33	33/33 (100%)	1	1	1
136	41	85.37	35	35/35 (100%)	1	1	1
149	41	82.93	34	34/34 (100%)	1	1	1
175	41	90.24	37	37/37 (100%)	1	1	1
181	41	90.24	37	37/37 (100%)	1	1	1
186	41	87.8	36	36/36 (100%)	1	1	1
190	41	87.8	36	36/36 (100%)	1	1	1
202	41	73.17	30	30/30 (100%)	1	1	1
220	41	82.93	34	34/34 (100%)	1	1	1
230	41	82.93	34	34/34 (100%)	1	1	1
234	41	87.8	36	35/36 (97%)	0.83	1	0.89

Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	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 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.