

Summary Report – Run 126 gastric HER2 IHC

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Overview

With the inclusion of trastuzumab in conventional treatment of HER2-overexpressing gastric cancers, the CPQA-AQCP performs two gastric HER2 immunohistochemical challenges per year. This survey consisted of a 45-core tissue microarray of gastric carcinomas. 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 excellent. No false-positive or false-negative staining was observed. Participant-specific feedback is below:

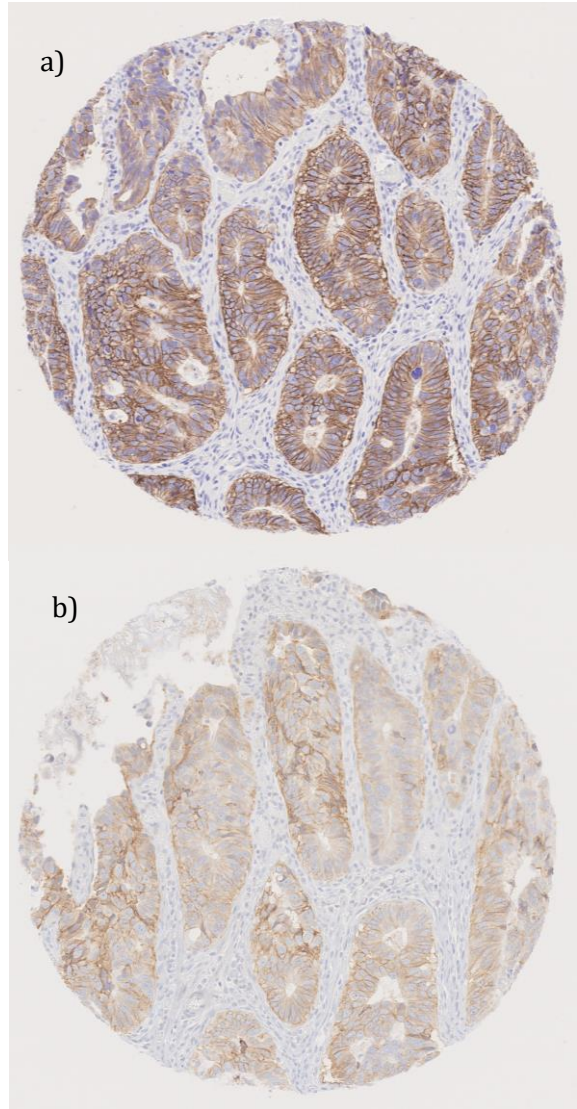
Lab ID	IHC Status*	Comment
101	Optimal	
111	Optimal	
114	Optimal	
136	Adequate	Artifactual staining of the luminal aspects of cells that may be attributed to use of the A0485 polyclonal
149	Optimal	
175	Optimal	
186	Optimal	Minor artifactual staining of the luminal aspects of cells that may be attributed to use of the A0485 polyclonal Ab
190	Optimal	
202	Adequate	Artifactual staining of the luminal aspects of cells that may be attributed to use of the A0485 polyclonal
207	Optimal	
220	Adequate	Weak and slight background
230	Optimal	

*based on CPQA assessor consensus

Garrattogram after CPQA assessment:

Lab/ Core	101	111	114	136	149	175	186	190	202	207	220	230	FISH
1	0	0	0	0	0	0	0	0	0	0	0	0	Neg
2	0	1	0	1	0	0	1	0	0	1	0	0	Neg
3	0	0	0	0	0	0	0	0	0	0	1	0	Equiv
4	0	0	0	0	0	0	0	0	0	0	0	0	Equiv
5	0	2	0	2	2	1	2	0	2	1	1	1	N/A
6	0	0	0	1	0	0	0	0	0	0	1	0	N/A
7	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	Amplified
9	U	U	U	U	U	U	U	U	U	U	U	U	Neg
10	U	U	U	U	U	U	U	U	U	U	U	U	Equiv
11	0	0	0	0	0	0	0	0	0	0	0	0	Equiv
12	U	1	0	1	1	1	0	0	0	1	0	1	Equiv
13	0	0	0	0	0	0	0	0	0	0	0	0	Neg
14	0	1	1	0	1	1	1	1	0	1	1	1	Equiv
15	1	2	1	1	1	2	2	1	0	2	0	2	Equiv
16	0	0	0	0	0	0	0	0	0	0	0	0	Neg
17	0	0	0	0	0	0	0	0	U	0	0	U	Neg
18	0	1	0	0	1	0	0	0	0	0	0	0	Neg
19	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	Amplified
21	0	0	0	0	0	0	0	0	0	0	0	0	Equiv
22	0	1	1	1	1	1	1	0	0	1	0	0	Equiv
23	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	Neg
25	0	0	0	0	0	0	0	0	0	0	0	0	Neg
26	0	0	0	0	0	0	0	0	U	0	U	0	Neg
27	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	Neg
29	0	0	0	0	0	0	0	0	0	0	0	0	Neg
30	0	1	0	0	0	1	0	0	0	0	1	1	Neg
31	3	3	3	3	3	3	3	3	3	3	2	3	Amplified
32	3	3	3	3	3	3	3	3	3	3	2	3	Amplified
33	0	1	0	0	0	0	0	0	0	0	0	0	Equiv
34	0	0	0	0	0	0	0	0	0	0	1	0	Equiv
35	0	1	0	1	1	1	2	0	1	2	1	1	Neg
36	0	1	0	0	1	1	1	0	0	2	0	1	Neg
37	0	0	0	1	0	0	0	0	0	0	0	1	Neg
38	0	0	0	0	0	0	0	0	0	0	0	0	N/A
39	0	0	0	0	0	0	0	0	0	0	0	0	N/A
40	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	Equiv
42	0	1	0	1	1	1	1	1	0	1	1	0	Neg
43	0	0	0	1	0	0	0	0	0	0	0	0	Neg
44	U	U	U	U	U	U	U	U	U	U	U	U	Neg
45	0	0	0	0	0	0	0	0	0	0	1	0	Neg

Figure 1. Representative images of a) optimal intensity staining and b) weak staining in Core 32.



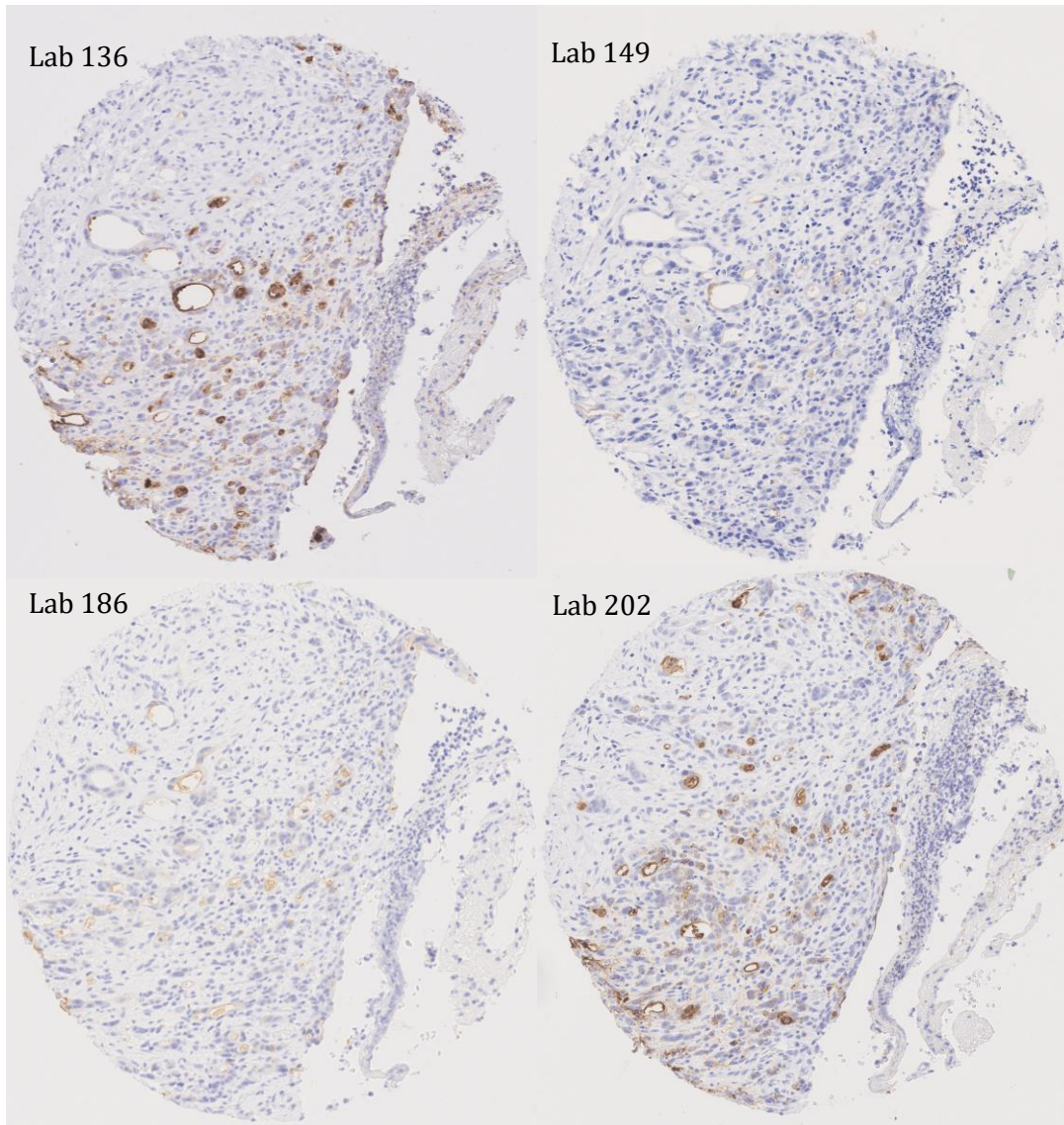


Figure 2. Representative images of artifactual staining in Core 29 that may be attributed to use of the A0485 rabbit polyclonal antibody, but can be limited with protocol adjustments as demonstrated by Lab 149 and Lab 186.

Supplementary Table 1 summarizes 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 contains scanned images that were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.

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)	Chromogen
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	4B5	1:8	ROCHE DIAGNOSTICS	F20661	15 MIN	DAKO Envision FLEX	N	N	DAB
111	Benchmark Ultra	IHC	HIER	36	4B5	predilute	Ventana	G20114	16	Ultraview	N	Y	DAB
114	Dako Omnis	LDT	Envision Flex TRS, high pH	30	4B5	1:8	Roche	G04523	25	Envision FLEX Dako Omnis	N	N	Envision FLEX DAB
136	DAKO AS480	HERCEP TEST	DAKO PT LOW PH	40	A0485	RTU	DAKO/AGILENT	20080444	30	ENVISION FLEX+	N	N	DAB
149	OMNIS	LDT	high pH 97 C	31	AO485	1:800	Agilent/Dako	20062529	20	EnVision Flex	No	No	DAB
175	Benchmark Ultra (Roche)	Roche	HIER	36	4B5	Pre-dilute	Roche	G23061	16	polymer ultra DAB	N	Y	DAB
186	LEICA BOND III	LDT	HIER	20	POLYCLONAL	1:400	DAKO	20073786	15	BOND POLYMER REFINE DETECTION	N	N	DAB
190	Ventana Benchmark Ultra	IHC Kit	Heat Retrieval	32	4B5	RTU	Roche Ventana	G20114	24	Optiview DAB	N	N	DAB
202	DAKO AUTOSTAINER	IHC KIT	HIER Ph 6.0	40	AO485	RTU	AGILENT	20080444	30	HECEPTEST KIT	N	N	DAB
207	BenchMark Ultra	LTD	CC1-Online	36 minutes	4B5	Prediluted	Ventana	G23061	16 minutes	UltraView	N	Y	DAB
220	BENCHMARK ULTRA	LDT	HIER	36	SP3	1/150	THERMO SCIENTIFIC	9103S1305H	28	VENTANA ULTRAVIEW	N	Y	DAB
230	Benchmark Ultra	LDT	HIER	32	4B5	predilute	Roche Diagnostics	G23061	16	Ultraview	N	N	DAB

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	86.67	39	39/39 (100%)	1	1	1
111	45	88.89	40	40/40 (100%)	1	1	1
114	45	88.89	40	40/40 (100%)	1	1	1
136	45	88.89	40	40/40 (100%)	1	1	1
149	45	88.89	40	40/40 (100%)	1	1	1
175	45	88.89	40	40/40 (100%)	1	1	1
186	45	88.89	40	40/40 (100%)	1	1	1
190	45	88.89	40	40/40 (100%)	1	1	1
202	45	84.44	38	38/38 (100%)	1	1	1
207	45	88.89	40	40/40 (100%)	1	1	1
220	45	86.67	39	39/39 (100%)	1	1	1
230	45	86.67	39	39/39 (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, Conditionally¹
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	FAIL²
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.