Summary Report – Run 115 CD117

Assessors: X Wei, B Wolber, J Won

Overview

CD117 (c-KIT) is commonly used for the identification of Gastrointestinal Stromal Tumours (GISTs) and considered a Class II marker since it is associated with the therapeutic agent, Gleevac. The survey consisted of 32 tissue cores of GISTs and non-GIST mesenchymal neoplasms. Strong staining of mast cells as well as weak staining endothelial cells were assessed, as positive staining is expected. No staining in smooth muscle is expected. Tumours without a mutation in the gene encoding the c-KIT protein account for a minority (5-10%) of GIST and are expected to be negative for c-KIT by immunohistochemistry. Such tumors typically stain positively for DOG1, which has been reported to be expressed in GISTs irrespective of mutation status *(Lopes LF, West RB, Bacchi LM, van de Rijn M, Bacchi CE. DOG1 for the diagnosis of gastrointestinal stromal tumor (GIST): comparison between 2 different antibodies. Applied Immunohistochemistry & Molecular Morphology. 2010 Jul 1;18(4):333-7)*, and consideration should be given to using this immunostain in tumours where the differential diagnosis includes GIST and CD117 staining is negative.

No established cut-off for positive versus negative staining was defined by the CPQA-AQCP, and participants were asked to simply score the tumour cells as positive or negative according to current practice at each institution. The scoring system that laboratories were asked to apply was:

- Positive (P) – definitive (diagnostic) staining of tumour cells; granular cytoplasmic staining with prominent staining of the cell border
- Negative (N) – no staining in tumour cells or staining below cut-off for positivity at your institution
- Unsatisfactory (U) – technical problem that makes interpretation impossible, such as core drop off or no tumour cells present

Results

The rate of unsatisfactory cores due to core dropout or no tumour present was higher than usual, but a sufficient number of cores still remained for assessment purposes. Core 9 was noted to be weakly positive, with more than half of the core missing for most participants. Core 13 was also noted to be weakly positive and with few tumour cells. Participant-specific feedback is below:

Lab	IHC Status*	Comments
101	Optimal	Slightly weak
102	Optimal	Nice staining
103	Optimal	Nice staining
111	Optimal	
112	Optimal	
114	Optimal	
120	Optimal	Slightly weak
129	Adequate	Weaker staining than other labs
138	Optimal	Slightly weak
144	Optimal	
147	Optimal	
149	Optimal	Slightly weak
151	Optimal	
186	Adequate	Slight background; unexplained weak false-positive in Core 26
198	Adequate	High background in some cores
207	Optimal	
230	Optimal	
234	Optimal	

*based on CPQA assessor consensus

Garrattogram after CPQA assessment:

Lab ID	101	102	103	111	112	114	120	129	138	144	147	149	151	186	198	207	230	234	**Diagnosis**
1	N	U	N	U	U	U	U	U	U	U	U	N	U	U	U	U	U	U	GIST
2	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
4	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
6	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
7	U	U	U	U	U	U	U	U	U	U	U	U	U	P	U	U	U	U	GIST
8	N	U	U	U	U	U	U	U	N	U	N	N	U	U	N	U	U	U	GIST
9	N	P	P	P	P	P	P	N	N	P	P	N	P	P	P	P	U	P	GIST
10	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
11	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
12	N	U	U	U	U	U	N	N	N	U	U	N	U	N	U	U	N	U	GIST
13	N	P	P	P	P	P	P	N	N	U	P	N	P	P	P	P	U	P	GIST
14	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
15	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
16	N	U	N	U	U	U	N	N	U	U	U	N	N	N	N	U	U	U	GIST
17	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST
18	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Synovial Sarcoma
19	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Synovial Sarcoma
20	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	High grade sarcoma NOS
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	MPNST
22	N	U	U	U	U	N	U	N	U	N	U	N	N	N	U	U	U	U	Synovial Sarcoma
23	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Synovial Sarcoma
24	N	U	U	U	U	U	N	N	U	U	U	N	N	N	U	U	U	U	MPNST
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	MPNST
26	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N	Leiomyosarcoma
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Maligant Solitary Fibrous Tumor
28	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Fibromatosis
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Leiomyosarcoma
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Leiomyosarcoma
31	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Leiomyosarcoma
32	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	GIST

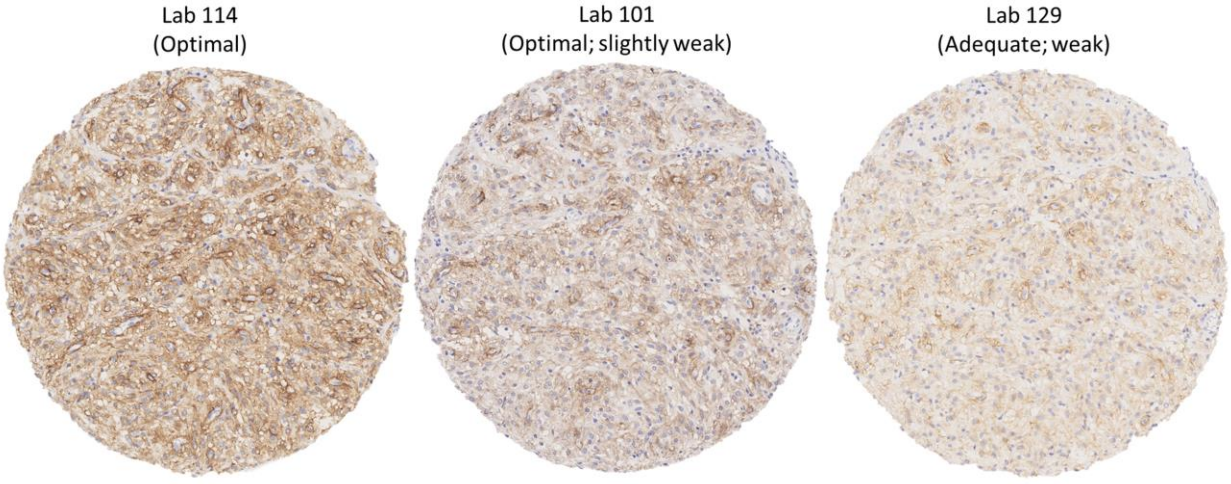

Figure 1. Representative images of the qualitative variability of positive CD117 staining.

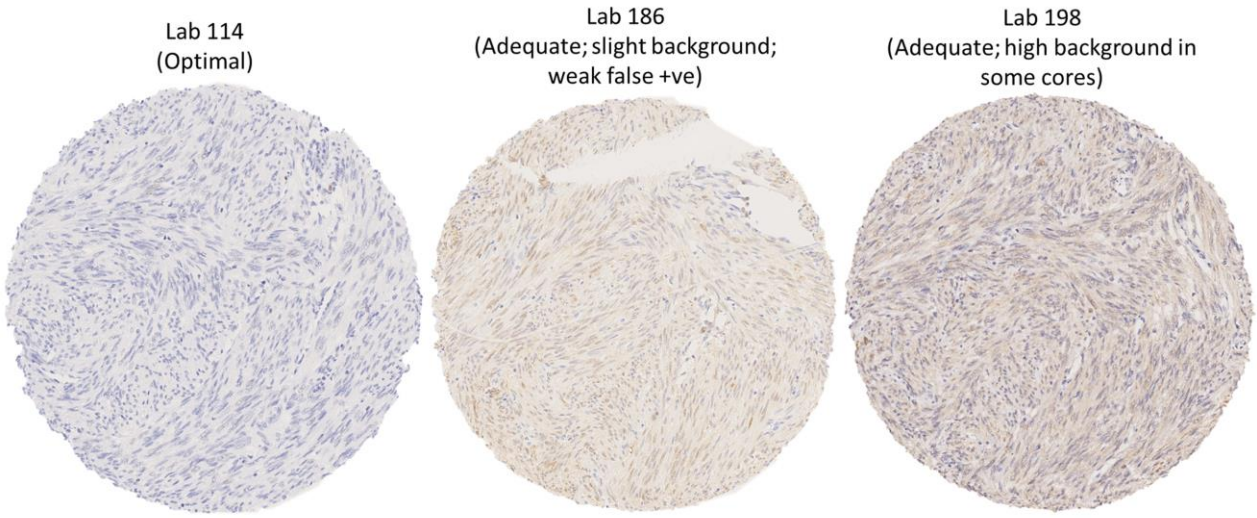

Figure 2. Representative images of the qualitative variability of CD117 IHC in a leiomyosarcoma (CD117-negative).

Supplementary Table 1 summarizes the reported staining protocols for CD117 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 CD117 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	n/a	EnVision FLEX	30 MIN	NA	1:200	DAKO	1E+07	30 MIN	DAKO FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retervial solution 9.0	20	YR145	1/50	Cell Marque	50407	30" RT	Dako Envision Flex	N	yes CuSO4
103	Benchmark Ultra	Commercial	CC1	48	Ckit YR145	Pre	Cell marque	50409	32	optiview	N	copper
111	BENCHMARK ULTRA	COMMERCIAL	HIER	48	YR145	100	CELL MARQUE	50407	32	OPTIVIEW	N	Y
112	BOND III	Commercial assay	BOND epitope retrieval 2 pH 9.0	18 minutes	EP10	RTU	LEICA	66507	15 minutes	BOND polymer refine DAB	no	no
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	polyclonal	1:200	Dako	1E+07	30	Envision FLEX DAKO Omnis	N	N
120	Autostainer Link48	LDT	HIER Waterbath	20	YR145	1:200	Cell Marque	50407	20	Dako Envision Flex	Y	N
129	Bond III	c-Kit	ER 2- High pH retrieval	20	Polyclonal Rabbit Anti-Huma	1:600	DAKO	1E+07	20	Bond Refine Detection Kit	N	N
138	Dako OMNIS	LDT	EDTA HIER	20	c-kit	1:400	Dako	1E+07	30	Polymer/Dako	N	N
144	Dako Omnis	LDT	Envision High Flex	30 min	Polyclonal Rabbit	1:200	Dako	1.1E+07	20 min	EnFlex Dako Omnis	No	Y
147	Leica Bond 3	LDT	ER 2 HIGH PH	20	C-KIT	300	DAKO	1E+07	15	Leica Refine Kit	N	N
149	Dako OMNIS	LDT	high pH OMNIS	20 min at 97 C	A4502	1:500	Dako Agilent	1E+07	20	EnVision Flex OMNIS	Yes	No
151	BOND-lll	commercial	HIER 2	20MIN	POLY	1:200	DAKO	1.1E+07	15 MIN	BOND REFINE	N	N
186	LEICA BOND III	LDT	HIER	20	CD117	1:250	DAKO	1E+07	15	BOND REFINE DE	N	N
198	DAKO OMNIS	LDT	HIER HIGH pH	30 @97deg	RABBIT POI	1/200	DAKO	1E+07	30	DAKO EnvisionHRP	N	N
207	BenchMark ultra	LDT	CC1-online	48 minutes	c-Kit	1/400	Dako	1E+07	220 minutes	Optiview DAB	N	Y
230	Benchmark Ultra	LDT	HEIR	40	YR145	NONE	CELL MARQU	52437	32	OPTIVIEW	N	N
234	Omnis	LDT	PT-module	30	YR145	RTU	CELL Marque	52437	25	DAKO Envision Flex	N	N

Table S2. Descriptive statistics based on CPQA assessment. Cores 9 and 13 were excluded from analyses for reasons noted above.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	30	90	23	23/23 (100%)	1	1	1
102	30	70	21	21/21 (100%)	1	1	1
103	30	76.67	21	21/21 (100%)	1	1	1
111	30	70	21	21/21 (100%)	1	1	1
112	30	70	21	21/21 (100%)	1	1	1
114	30	73.33	22	22/22 (100%)	1	1	1
120	30	80	22	22/22 (100%)	1	1	1
129	30	83.33	23	23/23 (100%)	1	1	1
138	30	76.67	21	21/21 (100%)	1	1	1
144	30	73.33	22	22/22 (100%)	1	1	1
147	30	73.33	21	21/21 (100%)	1	1	1
149	30	90	23	23/23 (100%)	1	1	1
151	30	80	23	23/23 (100%)	1	1	1
186	30	86.67	23	22/23 (96%)	1	0.92	0.91
198	30	76.67	21	20/21 (95%)	1	0.9	0.9
207	30	70	21	21/21 (100%)	1	1	1
230	30	73.33	21	21/21 (100%)	1	1	1
234	30	70	21	21/21 (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.