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Assessors' report for cIQc Run 76: MMR Immunohistochemistry

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Assessment performed on Friday, November 3, 2017 at Lion's Gate Hospital, North Vancouver, BC

Overview

Run 76 MMR (MLH1, PMS2, MSH2 and MSH6) consisted of a 40 single-core tissue microarray of colorectal carcinomas. MMR status was previously determined on whole sections using an IHC protocol validated against DNA sequencing. In the case of MMR protein immunohistochemistry, nuclear staining = **Expression**, which is normal and indicative of a non-mutant corresponding gene. **Absent** staining of the tumour cell nuclei, with positive staining of non-tumour cells, is an abnormal result. Note that absent staining is not always indicative of an underlying mutation (e.g. Lynch syndrome), but may be and warrants further testing. MMR immunohistochemistry is a screening test, not a definitive genetic test, and mutation status must be confirmed by DNA sequencing. A **failed** immunostain for MMR is when there is no staining of either tumour or normal cell nuclei, such that it is not possible to comment on MMR expression for that sample/stain.

In our experience an increased rate of "failed" results may be an indication of a technical lack of sensitivity of the test. For labs with many "failed" scores, interpretation may be correct in all cores that are interpretable but the screening for Lynch syndrome potentially becomes less accurate due to this poor sensitivity and increased likelihood of uninterpretable staining. Furthermore, MMR staining works best on well-fixed small biopsy specimens (e.g. colonic or endometrial biopsies). Hysterectomy/colonic resection specimens are usable but show heterogeneity of staining because of variable fixation. The cores for this TMA are from large resection specimens, and between the small core size and variable antigen preservation, are not optimal specimens for MMR assessment, corresponding to the most challenging samples we will encounter in practice. Core dropout or exhaustion of tumour within certain tissue microarray cores was a problem for this cIQc challenge

The MMR immunostains are always performed as part of a panel, and the biology of MMR protein expression can cause problems for interpretation. This is especially true for tumours with methylation of the promoter of MLH1, which can show complete loss of MLH1 and PMS2 expression, or patchy expression of either protein, and may even show patchy loss of MSH6 (as a secondary event, due to a hypermutable region in exon 5 of MSH6 that can become mutated as a result of MLH1 loss; Cores 10, 27 and 40). Weak MSH6 expression may also be associated with absence of expression of MSH2 (e.g. Cores 3, 8 and 11). In practice this is not a common phenomenon, as only rare cases show this combination of MSH2 loss of expression and weak/focal MSH6 expression. At this point we do not know if any of these patients have Lynch syndrome or whether this expression pattern is a result of epigenetic changes.

MLH1

MLH1 staining results were, overall, good. Considerably weaker staining by some labs was observed (relative to other participants). Lab 114 and 231 had particularly weak staining that made interpretation challenging or not possible for some cores (i.e. failed results). Participant-specific feedback is provided below:



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Lab ID	IHC Status*	Comments
101	Adequate	Weak
102	Optimal	Nice staining; patchy expression in Core 22 confirmed
103	Adequate	Weak; no internal control in Core 15
106	Optimal	Strong counterstain
107	Optimal	
109	Optimal	
110	Optimal	
111	Adequate	Weak
112	Optimal	
114	Sub-optimal	Very weak
115	--	Slide not available for assessment
116	--	Slide not available for assessment
125	Adequate	Weak
138	Optimal	
141	Optimal	
144	Optimal	
149	Optimal	
175	Adequate	Weak
181	Optimal	
186	Adequate	Slight background
189	Adequate	Weak
193	Adequate	Weak
194	Adequate	
202	Adequate	Some background
207	Adequate	Weak
217	Adequate	Some background
220	Adequate	Slight background; patchy expression in Core 22 confirmed
222	Optimal	
231	Sub-optimal	Very weak leading to several "Failed" cores due to lack of internal controls
236	Adequate	Weak

*based on CIQC assessment

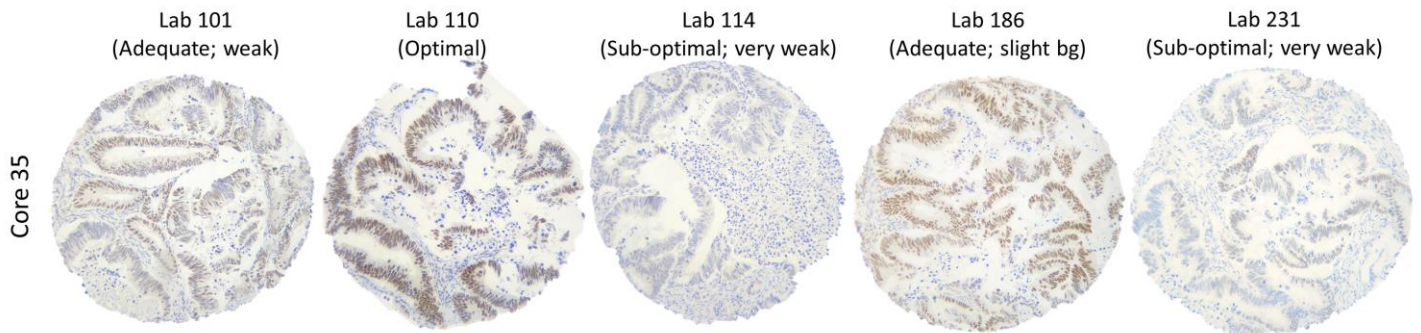


Figure 1. Representative staining of Core 35, a case expressing MLH1, by different participants. (bg = background)



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Lab/ Core	101	102	103	106	107	109	110	111	112	114	115	116	125	138	141	144	149	175	181	186	189	193	194	202	207	217	220	222	231	236	MMR Status		
1	U	U	U	U	E	U	U	E	U	U	U	U	E	U	U	U	U	U	E	U	E	U	A	U	A	E	E	U	U	U	MLH1		
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH6	
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	E	MSH2		
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	U	A	A	A	F	A	MLH1		
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	A	PMS2	
6	A	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	PMS2	
7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	A	U	A	A	A	A	A	MLH1	
8	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2	
9	U	A	A	A	A	U	A	A	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	A	A	A	A	A	A	MLH1	
10	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	MLH1	
11	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	MSH2	
12	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2	
13	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	MSH6	
15	E	E	F	E	E	F	E	E	E	F	F	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	F	F	MSH6	
16	U	A	A	A	A	A	A	A	U	A	U	A	A	A	A	A	A	U	A	U	A	A	A	U	A	A	A	A	F	U	A	MLH1	
17	U	U	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	A	U	A	A	A	A	A	MLH1	
18	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	MSH6	
19	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	U	A	A	A	A	A	A	MLH1	
20	U	U	U	U	U	U	U	U	U	A	U	U	A	A	A	A	A	A	A	A	U	U	U	A	U	U	U	U	U	U	A	MLH1	
21	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	F	A	MLH1	
22	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	MLH1	
23	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2	
24	A	U	U	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	U	A	U	U	A	A	A	A	MLH1	
25	A	U	U	A	A	A	A	A	A	A	A	A	A	U	A	U	A	A	A	U	U	U	A	U	A	A	A	A	A	A	A	PMS2	
26	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
27	A	A	F	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	F	A	MLH1	
28	U	U	U	U	U	U	U	U	U	E	U	U	E	E	E	E	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
29	U	E	U	E	U	U	E	U	U	U	U	U	U	U	U	U	E	U	E	U	E	U	U	U	U	E	U	U	U	U	U	MLH1	
30	U	U	U	U	U	U	U	U	U	U	E	U	E	U	U	U	U	E	U	A	U	U	U	U	E	U	U	U	U	U	U	MLH1	
31	U	U	E	E	U	E	E	E	U	E	E	E	E	U	E	E	U	E	U	E	U	U	U	U	E	E	U	E	U	U	PMS2		
32	A	A	A	A	A	A	U	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
33	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	U	U	A	A	A	A	A	PMS2	
34	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
35	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	U	E	U	E	E	E	E	E	E	MSH6	
36	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
37	U	U	U	U	U	A	U	A	A	U	A	A	A	U	A	A	A	A	A	U	U	U	U	U	A	A	A	A	A	A	A	MLH1	
38	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2
39	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1
40	U	A	A	A	A	A	A	A	U	U	A	U	U	U	U	U	U	U	A	A	A	A	U	U	U	A	A	A	F	U	A	MLH1	

PMS2: PMS2 staining results were very good, with all participants having either optimal or adequate results. Participant-specific feedback is provided below:



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Lab ID	IHC Status*	Comments
101	Adequate	Weak
102	Optimal	
103	Adequate	Slight background despite weak staining
106	Optimal	
107	Optimal	Very slight background
109	Adequate	Slight background
110	Optimal	
111	Adequate	Weak
112	Adequate	Slight background
114	Adequate	
115	--	Slide not available for assessment
116	--	Slide not available for assessment
125	Adequate	Slight background
138	Optimal	
141	Optimal	
144	Adequate	Slight background
149	Optimal	
175	Adequate	Background, as well as generally hazy staining
181	Optimal	
186	Optimal	
189	Optimal	
193	Adequate	Slight background
194	Adequate	Slight background and weak staining
202	Adequate	Strong background compared to others labs
207	Adequate	
217	Optimal	
220	Adequate	
222	Adequate	
231	Adequate	Slight background
236	Adequate	Slight background

*based on cIQc assessment

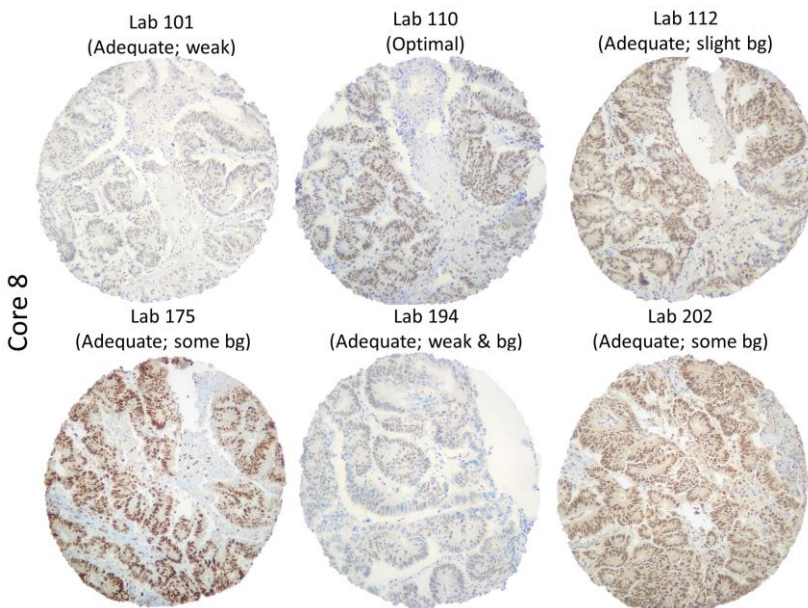


Figure 2. Representative staining of Core 8, a case expressing PMS2, by different participants. (bg = background)



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Lab/ Core	101	102	103	106	107	109	110	111	112	114	115	116	125	138	141	144	149	175	181	186	189	193	194	202	207	217	220	222	231	236	MMR Status	
1	U	U	U	U	E	U	U	E	U	U	E	E	U	U	U	U	U	U	U	U	U	U	U	U	A	E	E	U	U	U	MLH1	
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH6
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	MSH2	
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	MLH1	
5	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	PMS2	
6	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	PMS2	
7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	U	A	A	A	A	A	MLH1	
8	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2	
9	A	A	U	A	A	U	U	A	F	E	A	E	E	E	E	E	E	E	A	A	A	A	A	U	U	A	U	A	A	MLH1		
10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	A	A	A	A	A	A	MLH1	
11	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	MSH2	
12	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	PMS2	
13	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	E	U	U	U	MLH1		
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	F	E	MSH6	
15	E	E	F	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	F	E	F	MSH6	
16	A	A	U	U	A	A	A	U	A	A	A	A	F	A	A	A	U	E	A	A	A	A	A	U	A	A	U	A	U	A	MLH1	
17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	U	A	U	A	A	A	A	A	MLH1	
18	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	MSH6	
19	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	MLH1	
20	U	U	A	A	U	U	U	U	A	U	U	A	A	A	A	A	A	A	A	A	U	U	A	A	U	U	U	U	U	U	MLH1	
21	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
22	A	E	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	A	E	A	A	A	A	MLH1	
23	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2	
24	U	U	A	A	A	F	U	A	A	A	A	A	A	A	A	A	A	A	A	U	U	U	A	U	A	A	A	A	A	A	MLH1	
25	A	A	A	A	A	F	A	A	A	A	A	A	A	U	A	A	A	A	A	U	F	U	U	A	U	A	A	A	A	A	PMS2	
26	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
27	F	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
28	U	U	U	U	U	U	U	U	E	U	E	E	E	E	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
29	U	E	E	E	U	E	E	U	U	E	U	F	A	U	E	U	E	U	A	F	U	U	U	E	U	U	U	U	U	MLH1		
30	U	U	U	U	U	U	U	U	U	E	U	F	A	U	U	U	F	U	A	F	U	U	U	A	F	U	U	U	U	MLH1		
31	E	E	U	E	U	E	E	U	E	E	E	U	U	U	E	U	U	E	U	E	U	E	U	E	U	E	E	U	E	U	PMS2	
32	F	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
33	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	U	A	A	A	U	A	PMS2	
34	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1	
35	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	U	E	U	E	E	E	E	E	E	MSH6	
36	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
37	U	U	F	A	U	U	A	A	A	E	A	A	A	F	A	A	A	A	U	U	U	F	U	U	A	A	A	A	A	F	MLH1	
38	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2	
39	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
40	A	A	U	A	A	F	U	A	A	U	A	A	U	U	U	U	U	A	A	A	A	A	A	U	U	A	A	A	F	A	MLH1	

MSH2: More sub-optimal MSH2 staining results than previous runs were observed in this challenge. Participant-specific feedback is provided below:



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Lab ID	IHC Status*	Comments
101	Sub-optimal	Extremely weak leading to many failed cores
102	Optimal	
103	Adequate	Weak
106	Optimal	Nice staining
107	Borderline Adequate	Very weak
109	Optimal	
110	Optimal	
111	Adequate	Tissue appears overdigested
112	Optimal	
114	Adequate	Weak
115	--	Slide not available for assessment
116	--	Slide not available for assessment
125	Optimal	
138	Optimal	
141	Sub-optimal	Extremely weak leading to many failed cores
144	Adequate	Weak
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	High background
189	Adequate	Weak
193	Adequate	Strong counterstain with weak staining
194	Sub-optimal	Extremely weak leading to many failed cores
202	Adequate	Weak
207	Adequate	Weak
217	Adequate	Slight background
220	Adequate	Slight background
222	Optimal	
231	Adequate	Slight background
236	Adequate	Weak

*based on cIQc assessment



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Lab/ Core	101	102	103	106	107	109	110	111	112	114	115	116	125	138	141	144	149	175	181	186	189	193	194	202	207	217	220	222	231	236	MMR Status		
1	U	U	U	E	E	U	U	E	U	U	U	U	A	E	U	U	U	U	E	U	U	U	U	U	U	E	E	U	U	U	U	MLH1	
2	F	E	E	E	E	E	E	E	E	E	E	E	A	E	E	U	U	U	E	E	E	E	F	F	F	E	E	E	E	E	E	MSH6	
3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	MSH2	
4	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	F	U	E	E	E	E	E	MLH1	
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2	
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	PMS2	
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	MLH1	
8	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MSH2	
9	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	E	MLH1	
10	F	E	E	E	A	U	E	E	E	E	E	E	E	E	F	E	E	E	E	U	U	U	U	U	U	E	E	E	E	E	E	MLH1	
11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	U	A	A	A	A	A	A	MSH2	
12	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2	
13	U	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
14	F	F	F	F	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E	U	E	E	U	E	E	E	E	E	MSH6	
15	F	E	F	E	F	A	E	A	F	F	E	A	E	E	F	F	E	A	E	A	U	F	E	F	F	E	E	F	F	A	E	MSH6	
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20	U	U	U	U	F	U	U	U	U	U	U	U	U	E	E	E	E	E	E	E	U	E	E	E	U	E	U	U	U	U	E	MLH1	
21	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
22	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
23	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	F	A	A	A	A	A	A	MSH2	
24	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	U	E	E	E	E	E	E	MLH1	
25	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	E	PMS2	
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1	
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28	U	U	U	U	U	U	U	U	U	U	U	U	A	A	A	A	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
29	F	E	E	E	U	U	U	U	U	U	U	U	E	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
30	U	U	E	U	U	U	U	U	U	U	U	U	E	F	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
31	F	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	U	U	E	E	E	E	E	E	PMS2	
32	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
33	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2
34	E	E	E	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
35	F	U	U	F	F	A	A	A	F	F	F	A	A	E	F	F	E	A	A	A	U	F	U	F	U	A	F	A	F	A	E	MSH6	
36	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2
37	E	U	U	U	U	E	U	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
38	F	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MSH2
39	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1
40	F	E	E	E	E	E	E	E	E	E	E	E	U	U	U	U	U	U	E	E	E	U	E	U	U	E	E	E	E	E	E	MLH1	

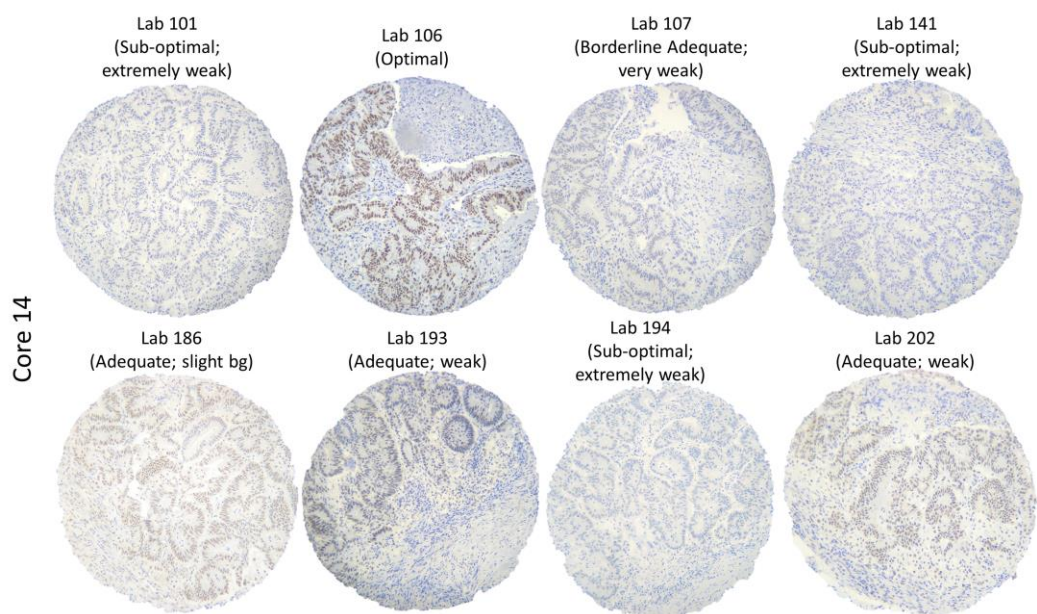


Figure 3. Representative staining of Core 14, a case expressing MSH2, by different participants. (bg = background)



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MSH6: MSH6 staining results were very good, overall, with only two sub-optimal results due to extremely weak staining (Lab 109) or significant cytoplasmic background (Lab 186). Cores 3 and 23 had variable and weak MSH6 weak staining that can be seen with MSH2 loss. Cores 11 and 27 were noted to have tumour sampling variability. Several labs had optimal staining for which no comments were noted and were, therefore, excluded from the list of participant specific feedback below:

Lab ID	IHC Status*	Comments
101	Adequate	Weak
102	Optimal	
103	Adequate	Weak
106	Optimal	
107	Adequate	Weak
109	Sub-optimal	Extremely weak
110	Optimal	
111	Optimal	
112	Optimal	
114	Adequate	Weak
115	--	Slide not available for assessment
116	--	Slide not available for assessment
125	Optimal	
138	Optimal	
141	Adequate	Weak
144	Adequate	Weak
149	Optimal	
175	Adequate	Weak
181	Optimal	
186	Sub-optimal	High cytoplasmic background that makes interpretation difficult
189	Optimal	
193	Optimal	
194	Adequate	Weak
202	Optimal	
207	Optimal	
217	Adequate	Slight background
220	Optimal	
222	Optimal	
231	Optimal	
236	Adequate	Weak and slight background

*based on CIQC assessment



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Lab/ Core	101	102	103	106	107	109	110	111	112	114	115	116	125	138	141	144	149	175	181	186	189	193	194	202	207	217	220	222	231	236	MMR Status	
1	U	U	U	U	U	U	U	E	U	U	E	E	U	U	U	U	U	U	E	U	U	U	U	U	E	E	E	E	U	U	MLH1	
2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MSH6
3	A	E	F	A	A	F	A	E	A	E	A	E	A	A	A	A	A	A	A	E	E	A	E	E	U	E	F	F	E	A	MSH2	
4	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	MLH1	
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2	
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	PMS2	
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	MLH1	
8	A	A	F	A	A	A	A	A	A	A	E	A	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	MSH2	
9	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	MLH1	
10	A	A	A	A	A	A	A	A	A	E	A	E	E	E	E	E	A	A	A	A	A	A	U	U	E	E	A	A	A	A	MLH1	
11	A	E	A	A	A	A	E	A	A	A	E	U	A	A	A	A	A	A	A	A	A	A	F	U	E	A	A	A	A	A	MSH2	
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14	A	A	F	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	MSH6	
15	F	A	F	A	F	F	A	A	A	F	F	A	A	A	F	F	A	A	A	A	A	F	F	A	A	A	F	F	F	A	MSH6	
16	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	U	E	U	E	U	E	U	E	E	E	MLH1	
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	U	E	U	E	E	E	E	MLH1	
18	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	U	A	A	A	A	A	MSH6	
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	U	E	E	E	E	E	E	MLH1	
20	F	U	A	U	F	U	U	U	U	U	U	E	U	E	E	E	E	E	E	U	U	E	E	E	U	E	U	U	U	U	MLH1	
21	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1	
22	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1	
23	A	A	A	A	A	F	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	MSH2	
24	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	U	E	E	E	E	E	E	MLH1	
25	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	U	E	U	E	E	E	E	PMS2	
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1	
27	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	E	A	A	A	A	A	MLH1	
28	U	U	U	U	U	U	U	U	A	U	U	A	A	A	A	A	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
29	F	E	A	A	U	A	A	E	U	U	U	E	U	A	U	A	U	A	A	U	A	A	U	U	U	A	U	U	U	U	MLH1	
30	U	U	E	U	U	U	U	U	U	U	E	U	F	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
31	E	E	E	E	U	E	E	U	E	E	E	U	E	U	E	U	E	U	E	U	E	E	E	U	U	E	U	U	U	U	PMS2	
32	E	E	E	E	E	F	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1	
33	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2	
34	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1	
35	U	A	F	U	F	A	A	A	F	F	F	A	A	A	F	F	A	A	A	U	U	F	U	A	U	A	F	A	A	A	MSH6	
36	U	U	U	U	U	U	U	U	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
37	E	U	E	U	U	E	U	E	E	E	E	E	E	E	E	E	U	E	E	U	U	U	U	U	E	E	E	E	E	E	MLH1	
38	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	MSH2	
39	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MLH1	
40	E	E	A	E	E	F	E	E	U	E	E	U	U	U	U	U	U	U	E	E	E	U	E	E	U	E	E	A	E	E	MLH1	

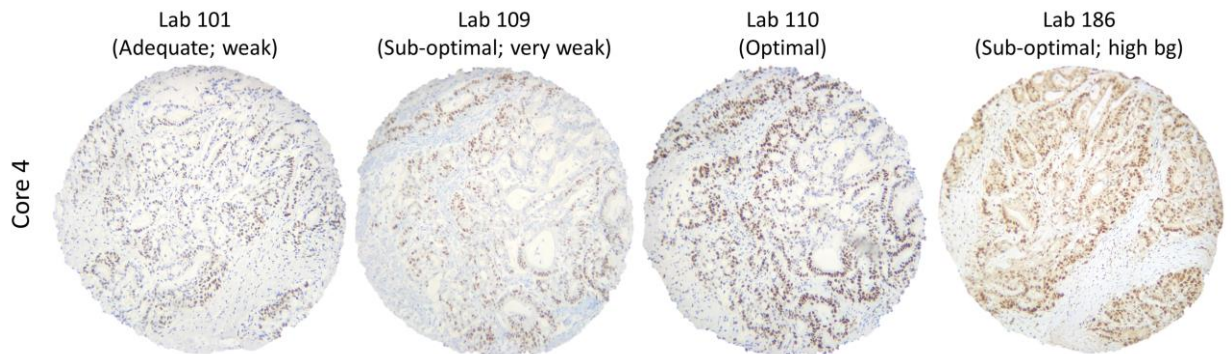


Figure 4. Representative staining of Core 4, a case expressing MSH6, by different participants. (bg=background)



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Supplementary Tables 1 to 4 summarizing staining protocols and Supplementary Tables 5 to 8 summarizing descriptive statistics can also be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with caution. Your regular 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 MLH1 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	CC1	64 min	ES05	1:50	Leica	6025230	32min	OptiView	N	N	DAB
102	DAKO PT - HIGH PH	20	ES05	1:80	DAKO	10115966	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
103	CC1	64	ESO5	1/20	LEICA	6048088	40	OPTIVIEW	N	Y	DAB
106	microwave pressure cooker	30	ESO5	1:105	Novocastra	44922	45	MACH4	no	no	DAB
107	ultra cc1	64	ES05	1:20	Leica/Novocastra	6042101	40	Optiview DAB	N	Y	DAB
109	HIER high pH(cc1)	32 min	M1	RTU	ROCHE	G07286	16 MIN	OPTIVIEW	N	Y	DAB
110	Dako PT high pH	20 min	ES05	1:100	DAKO	10121400	20 Min	DAKO Envision Flex Flex+20 Ms	Y	N	DAB
111	HIER	48	G168-15	1/50	BIOCARE	81716	32	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	25 minutes	ES05	1:100	Leica (Novocastra)	6042101	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	32	ES05	1/50	Leica	6023826	44	Optiview	N	Y	DAB
115	Envision Flex High pH	30 mins	ES05	RTU	Agilent	10120744	20 mins	Envision Flex	N	N	DAB
116	CC1	48 min	G168-15	1/80	BD Pharmingen	5329901	44 min	Optiview DAB	N	Y	DAB
138	HIER High pH	20	ES05	RTU	Dako	10120744	20	Dako Envision Flex +	Y	N	DAB
141	HIER	20	ES05	1:100	DAKO	10121400	20	POLYMER	Y	N	DAB
144	CC1	56 min	ES05	1:25	Novocastra	6039695	32 min.	Opti-View	No	Copper	DAB
149	PT Link high pH	20 min at 97 C	ES05	RTU	Dako Agilent	10122657	30	EnVision Flex	No	No	DAB
175	HIER	64	M1	Pre dilute	Roche	907286	16	opti view polymer	n	y	DAB
181	HIER pH10	20	ES05	1:100	DAKO	10121400	20	HRP Polymer	Y	N	DAB
186	HIER	20	G168-728	1:50	CELL MARQUE	15290303G	15	POLYMER	N	N	DAB
189	CC1	64	M1	pre-dilute	Ventana	unknown	24	OptiView DAB	N	N	OptiView DAB
193	HIER Low pH Omnis	30 Min.	ES05	RTU	DAKO	10111636	30 Min	Envision Flex	Yes	No	DAB
194	CC1	64	M1	PREDILUTE	VENTANA	G09887	16	OPTIVIEW	N	N	DAB
202	HIER citrate pH 9.5	20	es05	1/10	pharmagen BD	6140540	15 MIN	Leica Refine detection kit	no	no	dab
207	High PH	30 minutes	ES05	prediluted	Dako	1012575	20	Envision fLEX	Y	Y	DAB
217	HIER CC1	64	M1	RTU	Roche Ventana	G05543	60	Optiview	N	Y	DAB
220	CC1	72min	ES05	1/25	DAKO	10114422	1hr	OptiView	N	N	DAB
222	Ultra CC1	40	ES05	1:1	Ventana	G03827	4	Optiview DAB	Y	Y	Copper
231	ULTRA CC1	64 mins	M1	RTU	ROCHE/VENTANA	G03827	40 mins	ULTRAVIEW (VENTANA)	Y	N	DAB
236	CC1	64	ES05	1:20	DAKO	10100344	40	OptiView	N	N	DAB

Table S2. Reported PMS2 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	CC1	32 min	EP51	1:50	Dako	10125596	32min	OptiView	N	N	DAB
102	DAKO PT - HIGH PH	20	EP51	1:20	DAKO	10121526	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
103	CC1	64	EPR3947	PRE	CELL MARQUE	1506813B	1 HOUR	OPTIVIEW	Y	Y	DAB
106	microwave pressure cooker	30	MRQ-28	1:25	Cell Marque	1516709B	45	MACH4	no	no	DAB
107	Decloak FLEX TRS High pH	120celcius, 30seconds	A16-4	1:200	BD Pharmingen	6300808	30	FLEX 30+	N	N	DAB
109	HIER HIGH pH (CC1)	64 min	EPR3947	RTU	ROCHE	1626504C	20 MIN	OPTIVIEW	YES	YES	DAB
110	Dako PT high pH	20 min @ 97 C	EP51	1:50	DAKO	10117823	30	DAKO Envision flex-Flex 30 DD	N	N	DAB
111	HIER	48	EP51	1/100	DAKO	10122891	32	OPTIVIEW	Y	Y	DAB
112	Bond Epitope Retrieval 2 pH 9.0	30 minutes	EP51	1:75 using DAKO background reducing diluent	DAKO	10114768	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	64	EP51	1/25	Epitomics	10122891	64	Optiview	N	Y	DAB
115	Envision Flex High pH	30 mins	EP51	RTU	Agilent	10119098	30 mins	Envision Flex	N	N	DAB
116	CC1	64 min	EPR3947	RTU	Ventana	1626504 B	60 min	Optiview DAB	Y	Y	DAB
138	HIER High pH	20	EP51	RTU	Dako	10120132	30	Dako Envision Flex +	N	N	DAB
141	HIER	20	EP51	1:50	DAKO	10117823	30	POLYMER	N	N	DAB
144	CC1	64 min	EPR3947	Pre-Dilute	Cell Marque	1626504D	60 min	Opti-View	No	Copper	DAB
149	PT Link high pH	20 min at 97 C	EP51	RTU	Dako Agilent	10122662	20	EnVision Flex	Yes	No	DAB
175	HIER	64	EPR 3947	Pre dilute	Roche	1626504E	32	opti-kit polymer	y	y	DAB
181	HIER pH 10	20	EP51	1:50	DAKO	10117823	30	HRP Polymer	N	N	DAB
186	HIER	20	ERP3947	1:4	CELL MARQUE	1532405J	15	POLYMER	N	N	DAB
189	CC1	92	A16-4	pre-dilute	Ventana	unknown	32	OptiView DAB	Y	N	OptiView DAB
193	HIER High pH Omnis	30 Min.	EP51	RTU	DAKO	10114394	30 Min	Envision Flex	Yes	No	DAB
194	CC1	64	EPR3947	PREDILUTE	VENTANA	V0000163	32	OPTIVIEW	N	N	DAB
202	HIER citrate pH 9.5	30	a16-4	1/25	pharmagen BD	6334913	15 MIN	Leica Refine detection kit	no	no	DAB
207	High Ph	30 miutes	EP51	prdilute	Dako	10124745	20 miutes	Envision Flex	N	Y	DAB
217	HIER CC1	40	EPR3947	RTU	Roche Ventana	1626504E	48	Optiview	Y	Y	DAB
220	CC1	64min	EP51	1/40	DAKO	10112572	1h8min	OptiView	N	N	DAB
222	Ultra CC1	92	EPR3947	1:1	Ventana	1626504C	32	Optiview DAB	Y	Y	Copper
231	ULTRA CC1	64 mins	EP51	1/25	DAKO	10117823	80 mins	ULTRAVIEW (VENTANA)	Y	N	DAB
236	CC1	64	EPR3947	RTU	Ventana/ROCHE	1506813C	48	OptiView	N	N	DAB

Table S3. Reported MSH2 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	CC1	32 min	G219-1129	1:200	Cell Marque	1313003B	32min	OptiView	N	N	DAB
102	DAKO PT - HIGH PH	20	FE11	1:40	DAKO	10114761A	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
103	CC1	56 MINS	G219-1129	PRE	CELL MARQUE	1417104F	32 MINS	OPTIVIEW	N	Y	DAB
106	microwave pressure cooker	30	FE11	1:40	Dako	10122595	45	MACH4	no	no	DAB
107	cc1	32	G219-1129	1:200	Cell Marque	1505809A	32	Optiview DAB	N	Y	DAB
109	HIER HIGH pH(cc1)	32 min	G219-1129	RTU	ROCHE	1616008D	8 MIN	OPTIVIEW	N	Y	DAB
110	Dako PT high pH	20 min @ 97 C	FE11	1:150	DAKO	10114761A	20 Min	DAKO Envision flex-Flex+20 Ms DD	Y	N	DAB
111	HIER	40	G219-1129	1/600	CELL MARQUE	1616010A	32	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	30 minutes	FE11	1:100	DAKO	10117989	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	32	G219-1129	1/100	Cell Marque	1505809B	32	Optiview	N	Y	DAB
115	Envision Flex High pH	30 mins	FE11	RTU	Agilent	10120750	20 mins	Envision Flex	N	N	DAB
116	CC1	40 min	G219-1129	1/400	Cell Marque	1505809 F	48 min	Optiview DAB	N	Y	DAB
138	HIER High pH	20	FE11	RTU	Dako	10124949	20	Dako Envision Flex +	Y	N	DAB
141	HIER	20	FE11	1:150	DAKO	10114761A	20 min	POLYMER	Y	N	DAB
144	CC1	40 min	G219-1129	Pre-Dilute	Cell Marque	1529502G	16 min.	Opti-View	No	Copper	DAB
149	PT Link high pH	20 min at 97 C	FE11	RTU	Dako Agilent	10120750	20	EnVision Flex	Yes	No	DAB
175	HIER	32	G219-1129	pre-dilute	Rohce	1616008	16	opti-view polymer	n	y	DAB
181	HIER pH 10	20	FE11	1:150	DAKO	10114761A	20	HRP Polymer	Y	N	DAB
186	HIER	20	G219-1129	1:200	CELL MARQUE	1529504A	15	POLYMER	N	N	DAB
189	CC1	40	G219-1129	pre-dilute	Ventana	unknown	12	OptiView DAB	N	N	OptiView DAB
193	HIER High pH Omnis	30 Min.	FE11	RTU	DAKO	10112557	30 Min	Envision Flex	No	No	DAB
194	CC1	32	G219-1129	PREDILUTE	VENTANA	V0000154	16	OPTIVIEW	N	N	DAB
202	HIER citrate pH 9.5	20	25d12	1/50	Leica	6049441	15 MIN	Leica Refine detection kit	no	no	dab
207	High PH	30 miutes	FE11	prediluted	Dako	10124949	30	Envision flex	N	Y	DAB
217	HIER CC1	56	G219-1129	RTU	Roche Ventana	1529502H	32	Optiview	N	Y	DAB
220	CC1	32min	G219-1129	Pre-Dilute	Cell Marque	1616008B	32min	OptiView	N	N	DAB
222	Ultra CC1	64	G219-1129	1:1	Ventana	1529502G	24	Optiview DAB	Y	Y	Copper
231	ULTRA CC1	64 mins	G219-1129	RTU	CELL MARQUE	1529502 C	20 mins	ULTRAVIEW (VENTANA)	Y	N	DAB
236	CC1	40	G219-1129	RTU	Ventana/ROCHE	1529502C	20	OptiView	N	N	DAB

Table S4. Reported MSH6 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	CC1	32 min	EP49	1:50	Epitomics	EN072101	32min	OptiView	N	N	DAB
102	DAKO PT - HIGH PH	20	EP49	1:100	DAKO	10116602	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
103	CC1	64	44	PRE	VENTANA	G05536	1 HOUR	OPTIVIEW	N	Y	DAB
106	microwave pressure cooker	30	SP93	1:20	Cell Marque	1625006b	60	MACH4	no	no	DAB
107	ultra cc1	56	EPR 3945	1:750	Abcam	GR262215-11	32	Optiview DAB	N	Y	DAB
109	HIER HIGH pH(CC1)	64 MIN	44	RTU	ROCHE	G07632	20 MIN	OPTIVIEW	N	Y	DAB
110	Dako PT high pH	20 min @ 97 C	EP49	1:200	DAKO	10116602	30	DAKO Envision Flex Flex 30	N	N	DAB
111	HIER	48	SP93	1/100	CELL MARQUE	1512802B	32	OPTIVIEW	N	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	40 minutes	EP49	1:1500	30 minutes	EN072101	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	64	EP49	1/200	Epitomics	ED072101	32	Optiview	N	Y	DAB
115	Envision Flex High pH	30 mins	EP49	RTU	Agilent	10119097	20 mins	Envision Flex	N	N	DAB
116	CC1	32 min	BC/44	1/400	Biocare Medicales	81916	48 min	Optiview DAB	N	Y	DAB
138	HIER High pH	20	EP49	RTU	Dako	10121735	20	Dako Envision Flex +	N	N	DAB
141	HIER	20	EP49	1:200	DAKO	10116602	30 min	POLYMER	N	N	DAB
144	CC1	32 min	EP49	1:100	EPTOMICS	20910	32 min	Opti-View	No	Copper	DAB
149	PT Link high pH	20 min at 97 C	EP49	RTU	Dako Agilent	10121735	20	EnVision Flex	No	No	DAB
175	HIER	64	44	1 in 100	Cell Marque	1112208C	24	opti view polymer	y	y	DAB
181	HIER pH 10	20	EP49	1:200	DAKO	10116602	30	HRP Polymer	N	N	DAB
186	HIER	20	BC/44	1:50	BIOCARE MEDICAL	110315	15	POLYMER	N	N	DAB
189	CC1	64	SP93	pre-dilute	Ventana	unknown	12	OptiView DAB	N	N	OptiView DAB
193	HIER High pH Omnis	30 Min.	EP49	RTU	DAKO	10114392	20 Min.	Envision Flex	No	No	DAB
194	CC1	64	44	PREDILUTE	VENTANA	G07632	16	OPTIVIEW	N	N	DAB
202	HIER citrate pH 9.5	40 min	pu29	1/25	ABCAM	GR262215	15 MIN	Leica Refine detection kit	no	no	dab
207	High PH	30 minutes	EP49	prdiluted	Dako	10123749	20 miutes	Envision fLEX	N	Y	DAB
217	HIER CC1	64	44	RTU	Roche Ventana	G04117	36	Optiview	N	Y	DAB
220	CC1	32min	EP49	1/50	DAKO	10100348	28min	OptiView	N	N	DAB
222	Ultra CC1	56	44	1:1	Ventana	G04117	16	Optiview DAB	Y	Y	Copper
231	ULTRA CC1	64 mins	EP49	1/25	DAKO	10116602	32 mins	ULTRAVIEW (VENTANA)	Y	N	DAB
236	CC1	40	EP49	1:50	DAKO	10100348	40	OptiView	N	N	DAB

Table S5. Descriptive statistics for MLH1 based on cIQc assessment (cores 1, 29 and 30 were excluded due to patchy expression).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	37	67.57	25	24/25 (96%)	0.92	1	0.92
102	37	70.27	26	25/26 (96%)	1	0.93	0.92
103	37	67.57	25	25/25 (100%)	1	1	1
106	37	81.08	30	30/30 (100%)	1	1	1
107	37	83.78	31	30/31 (97%)	1	0.94	0.93
109	37	75.68	28	28/28 (100%)	1	1	1
110	37	81.08	30	30/30 (100%)	1	1	1
111	37	83.78	31	31/31 (100%)	1	1	1
112	37	81.08	30	30/30 (100%)	1	1	1
114	37	75.68	28	27/28 (96%)	1	0.93	0.93
115	37	78.38	29	29/29 (100%)	1	1	1
116	37	86.49	32	32/32 (100%)	1	1	1
125	37	89.19	33	33/33 (100%)	1	1	1
138	37	86.49	32	32/32 (100%)	1	1	1
141	37	81.08	30	30/30 (100%)	1	1	1
144	37	89.19	33	33/33 (100%)	1	1	1
149	37	86.49	32	32/32 (100%)	1	1	1
175	37	78.38	29	29/29 (100%)	1	1	1
181	37	89.19	33	33/33 (100%)	1	1	1
186	37	83.78	31	31/31 (100%)	1	1	1
189	37	75.68	28	28/28 (100%)	1	1	1
193	37	59.46	22	22/22 (100%)	1	1	1
194	37	45.95	17	17/17 (100%)	1	1	1
202	37	70.27	26	26/26 (100%)	1	1	1
207	37	43.24	16	16/16 (100%)	1	1	1
217	37	81.08	30	30/30 (100%)	1	1	1
220	37	83.78	31	31/31 (100%)	1	1	1
222	37	83.78	31	31/31 (100%)	1	1	1
231	37	67.57	25	25/25 (100%)	1	1	1
236	37	78.38	29	29/29 (100%)	1	1	1

Table S6. Descriptive statistics for PMS2 based on cIQc assessment (core 9 was excluded due to patchy expression).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	39	61.54	24	23/24 (96%)	1	0.93	0.92
102	39	76.92	30	28/30 (93%)	1	0.89	0.86
103	39	69.23	27	27/27 (100%)	1	1	1
106	39	82.05	32	31/32 (97%)	1	0.95	0.93
107	39	79.49	31	31/31 (100%)	1	1	1
109	39	69.23	27	26/27 (96%)	1	0.94	0.92
110	39	74.36	29	28/29 (97%)	1	0.94	0.93
111	39	82.05	32	31/32 (97%)	1	0.95	0.93
112	39	71.79	28	28/28 (100%)	1	1	1
114	39	82.05	32	29/32 (91%)	1	0.86	0.8
115	39	82.05	32	31/32 (97%)	1	0.95	0.93
116	39	87.18	34	33/34 (97%)	1	0.95	0.94
125	39	79.49	31	31/31 (100%)	1	1	1
138	39	69.23	27	27/27 (100%)	1	1	1
141	39	82.05	32	30/32 (94%)	0.85	1	0.87
144	39	84.62	33	32/33 (97%)	1	0.95	0.94
149	39	82.05	32	32/32 (100%)	1	1	1
175	39	74.36	29	29/29 (100%)	1	1	1
181	39	84.62	33	31/33 (94%)	1	0.91	0.87
186	39	69.23	27	27/27 (100%)	1	1	1
189	39	69.23	27	24/27 (89%)	0.8	0.94	0.76
193	39	61.54	24	24/24 (100%)	1	1	1
194	39	66.67	26	25/26 (96%)	1	0.94	0.92
202	39	66.67	26	24/26 (92%)	1	0.88	0.84
207	39	43.59	17	16/17 (94%)	0.86	1	0.88
217	39	89.74	35	32/35 (91%)	0.93	0.9	0.82
220	39	76.92	30	29/30 (97%)	1	0.95	0.93
222	39	76.92	30	30/30 (100%)	1	1	1
231	39	69.23	27	26/27 (96%)	1	0.94	0.92
236	39	71.79	28	28/28 (100%)	1	1	1

Table S7. Descriptive statistics for MSH2 based on cIQc assessment (core 15 was excluded from analysis due to sampling variability).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	39	51.28	20	19/20 (95%)	0.94	1	0.77
102	39	71.79	28	28/28 (100%)	1	1	1
103	39	69.23	27	27/27 (100%)	1	1	1
106	39	79.49	31	31/31 (100%)	1	1	1
107	39	74.36	29	27/29 (93%)	0.92	1	0.79
109	39	71.79	28	27/28 (96%)	0.96	1	0.89
110	39	79.49	31	30/31 (97%)	0.96	1	0.89
111	39	82.05	32	31/32 (97%)	0.96	1	0.89
112	39	74.36	29	29/29 (100%)	1	1	1
114	39	76.92	30	30/30 (100%)	1	1	1
115	39	76.92	30	30/30 (100%)	1	1	1
116	39	84.62	33	30/33 (91%)	0.89	1	0.72
125	39	84.62	33	32/33 (97%)	0.96	1	0.9
138	39	87.18	34	34/34 (100%)	1	1	1
141	39	58.97	23	21/23 (91%)	0.89	1	0.78
144	39	79.49	31	31/31 (100%)	1	1	1
149	39	84.62	33	33/33 (100%)	1	1	1
175	39	71.79	28	27/28 (96%)	0.96	1	0.89
181	39	87.18	34	33/34 (97%)	0.97	1	0.87
186	39	74.36	29	28/29 (97%)	0.96	1	0.89
189	39	71.79	28	28/28 (100%)	1	1	1
193	39	64.1	25	25/25 (100%)	1	1	1
194	39	58.97	23	23/23 (100%)	1	1	1
202	39	61.54	24	24/24 (100%)	1	1	1
207	39	46.15	18	18/18 (100%)	1	1	1
217	39	87.18	34	33/34 (97%)	0.97	1	0.89
220	39	79.49	31	31/31 (100%)	1	1	1
222	39	76.92	30	29/30 (97%)	0.96	1	0.89
231	39	74.36	29	29/29 (100%)	1	1	1
236	39	74.36	29	28/29 (97%)	0.96	1	0.89

Table S8. Descriptive statistics for MSH6 based on cIQc assessment (cores 3, 8 and 11 were excluded due to weak expression associated with loss of MSH2; cores 10 and 29 were excluded due to observed patchy loss as a result of MLH1 loss).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	35	71.43	25	25/25 (100%)	1	1	1
102	35	77.14	27	27/27 (100%)	1	1	1
103	35	71.43	25	23/25 (92%)	0.9	1	0.75
106	35	74.29	26	26/26 (100%)	1	1	1
107	35	68.57	24	24/24 (100%)	1	1	1
109	35	60	21	21/21 (100%)	1	1	1
110	35	77.14	27	27/27 (100%)	1	1	1
111	35	80	28	28/28 (100%)	1	1	1
112	35	74.29	26	26/26 (100%)	1	1	1
114	35	77.14	27	26/27 (96%)	0.95	1	0.9
115	35	77.14	27	27/27 (100%)	1	1	1
116	35	88.57	31	31/31 (100%)	1	1	1
125	35	80	28	27/28 (96%)	0.95	1	0.92
138	35	85.71	30	29/30 (97%)	0.95	1	0.92
141	35	71.43	25	24/25 (96%)	0.95	1	0.88
144	35	77.14	27	26/27 (96%)	0.95	1	0.9
149	35	82.86	29	28/29 (97%)	0.95	1	0.92
175	35	68.57	24	24/24 (100%)	1	1	1
181	35	88.57	31	31/31 (100%)	1	1	1
186	35	71.43	25	25/25 (100%)	1	1	1
189	35	68.57	24	24/24 (100%)	1	1	1
193	35	71.43	25	25/25 (100%)	1	1	1
194	35	57.14	20	20/20 (100%)	1	1	1
202	35	71.43	25	23/25 (92%)	1	0.75	0.8
207	35	48.57	17	16/17 (94%)	1	0.8	0.85
217	35	88.57	31	31/31 (100%)	1	1	1
220	35	77.14	27	27/27 (100%)	1	1	1
222	35	77.14	27	26/27 (96%)	0.95	1	0.91
231	35	77.14	27	27/27 (100%)	1	1	1
236	35	74.29	26	26/26 (100%)	1	1	1