Assessors' report for cIQc Run 84: MMR Immunohistochemistry

Assessors: B Gilks, J Garratt, and J Won (recorder)

Assessment performed on June 27, 2018 and August 20, 2018 at Vancouver General Hospital, Vancouver, BC

Overview

Run 84 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 also 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 (e.g. Core 9), 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 very good. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
102	Optimal	Notably nice staining
106	Optimal	
107	Optimal	
109	Adequate	Generally weak staining
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	Slightly weak
125	Optimal	Slightly weak
138	Optimal	

Lab ID	IHC Status*	Comments
138	Optimal	
141	Optimal	
144	Optimal	
149	Optimal	
175	Adequate	Background blush in some cores; generally weak staining
181	Optimal	
186	Adequate	Cytoplasmic background in several cores
190	Optimal	
194	--	Slide not available
202	Optimal	
207	Optimal	
217	Adequate	General background blush
220	Optimal	
222	Optimal	
231	Optimal	

*based on cIQc assessment

Garrattogram after cIQc assessment of MLH1 (with unsatisfactory cores removed if present in more than 50% of labs):

Lab/ Core	102	106	107	109	110	111	112	114	125	138	141	144	149	175	181	186	190	194	202	207	217	220	222	231	MMR Status
1	E	E	E	U	E	E	U	E	E	U	U	U	U	U	U	E	U	U	E	U	E	E	E	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	MSH6
3	E	E	E	E	E	E	E	U	U	E	E	E	E	E	U	E	E	U	U	U	U	U	E	U	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	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	PMS2
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	PMS2
7	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	U	A	U	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	MSH2
9	U	U	E	U	E	E	A	A	A	U	A	E	A	A	U	A	A	A	A	A	A	A	A	A	MLH1
10	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	U	F	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	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	PMS2
14	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	MSH6
15	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
16	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
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	MSH6
19	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
20	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
21	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	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	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	MSH2
26	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
27	F	A	A	A	A	A	F	F	F	A	A	A	A	A	F	A	A	F	A	A	A	A	A	F	MLH1
32	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	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	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	MLH1
35	E	E	E	E	E	E	E	U	E	E	E	E	U	U	E	U	U	U	U	U	U	U	E	U	MSH6
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	MSH2

PMS2: PMS2 staining results were good overall, but sub-optimal results were observed in a handful of labs. Lab 111 had particularly weak staining that made interpretation very challenging. Conversely, Labs 190 and 222 had very intense staining and heavy background. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
102	Optimal	Slightly weak
106	Optimal	
107	Optimal	
109	Adequate	Generally weak staining
110	Optimal	Strong counterstain
111	Sub-optimal	Very weak staining
112	Adequate	General background blush
114	Adequate	Generally weak staining
125	Optimal	
138	Optimal	
141	Optimal	Slightly weak
144	Optimal	Slightly weak
149	Optimal	
175	Adequate	Background blush; generally weak staining
181	Optimal	Strong counterstain
186	Optimal	Slight background blush in some cores
190	Sub-optimal	Very intense staining; heavy background
194	--	Slide not available
202	Borderline Adequate	Generally strong background
207	Optimal	
217	Optimal	Slight background blush in some cores
220	Adequate	Background blush; generally weak staining
222	Sub-optimal	Very intense staining; heavy background
231	Adequate	Generally weak staining

*based on cIQc assessment

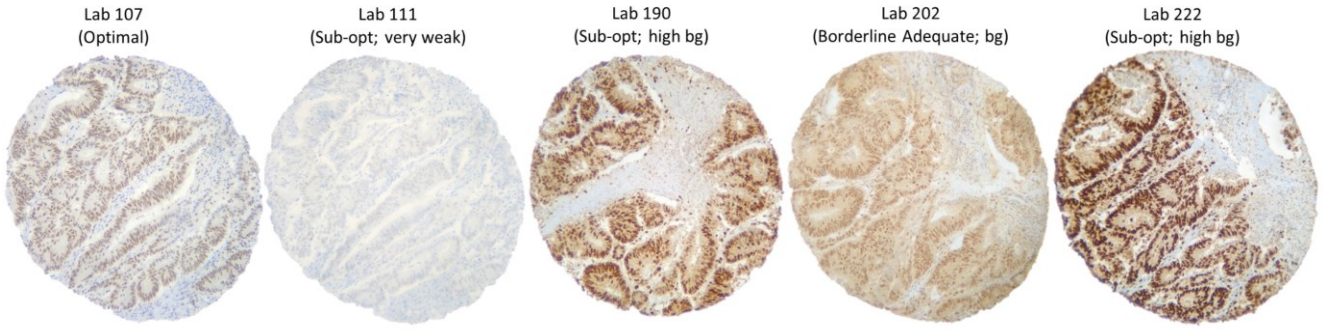

Figure 1. Representative images of Core 8, a case expressing PMS2, by different participants. (bg = background)

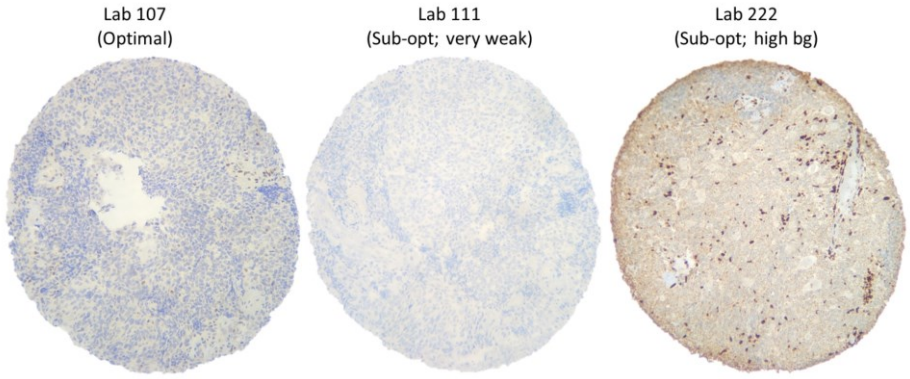

Figure 2. Representative images of Core 6, a case lacking PMS2 expression, by different participants. (bg = background)

Garrattogram after cIQc assessment of PMS2 (with unsatisfactory cores removed if present in more than 50% of labs):

Lab/ Core	102	106	107	109	110	111	112	114	125	138	141	144	149	175	181	186	190	194	202	207	217	220	222	231	MMR Status
1	E	E	E	U	E	E	U	E	E	U	U	U	U	U	U	E	U	U	E	U	E	E	E	E	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	MSH6
3	E	E	E	E	E	E	E	U	U	E	E	E	E	E	U	E	E	U	U	U	U	U	E	U	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	MLH1
5	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	PMS2
6	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	PMS2
7	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	U	A	A	U	A	U	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	MSH2
9	E	E	E	U	E	E	E	E	A	U	U	U	A	U	U	A	A	A	U	U	U	A	U	U	MLH1
10	A	A	A	A	A	F	A	F	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	F	MLH1
11	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2
12	A	A	A	A	A	A	A	F	A	A	A	A	U	A	A	A	E	A	A	A	A	A	A	A	PMS2
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	MSH6
15	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	MSH6
16	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
18	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	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	A	A	MLH1
20	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
21	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	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1
23	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	MSH2
26	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
27	A	A	A	F	A	A	F	F	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	F	MLH1
32	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	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	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	F	MLH1
35	E	E	E	E	E	E	E	U	E	E	E	E	U	U	E	U	U	U	U	U	U	U	E	U	MSH6
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	MSH2

MSH2: MSH2 staining results were very good overall. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
102	Optimal	
106	Optimal	
107	Optimal	Slightly weak
109	Adequate	Generally weak staining
110	Optimal	
111	Adequate	Generally weak staining
112	Optimal	
114	Adequate	Generally weak staining
125	Optimal	
138	Optimal	
141	Adequate	Generally weak staining; strong counterstain
144	Optimal	
149	Optimal	
175	Optimal	Slight background blush in some cores
181	Optimal	Strong counterstain
186	Optimal	
190	Optimal	
194	--	Slide not available
202	Borderline Adequate	Generally strong background
207	Optimal	Slightly weak
217	Optimal	Slight background blush in some cores
220	Optimal	
222	Optimal	
231	Optimal	Slightly weak

*based on cIQc assessment

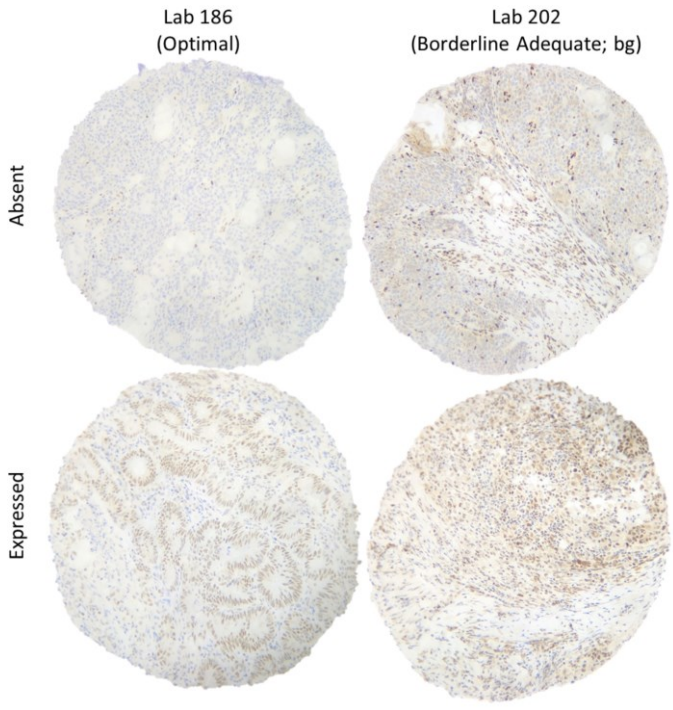

Figure 3. Representative staining in a case without MSH2 expression and a case with MSH2 expression in labs with similar staining protocols. (bg = background)

Garrattogram after cIQc assessment of MSH2 (with unsatisfactory cores removed if present in more than 50% of labs):

Lab/ Core	102	106	107	109	110	111	112	114	125	138	141	144	149	175	181	186	190	194	202	207	217	220	222	231	MMR Status
1	U	U	E	U	E	U	U	E	E	E	U	U	U	U	E	E	U	U	E	E	E	E	E	E	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	MSH6
3	A	A	A	A	A	A	A	U	U	A	A	A	A	A	U	A	A	U	U	U	A	U	A	U	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	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	PMS2
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	PMS2
7	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	U	E	E	U	E	U	MLH1
8	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
10	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	U	E	MLH1
11	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
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	PMS2
14	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	U	MSH6
15	E	E	F	F	E	F	E	F	F	E	F	E	E	F	E	F	F	A	E	E	E	F	F	F	MSH6
16	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
18	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	U	E	E	E	E	E	E	E	MSH6
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	MLH1
20	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
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	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	MLH1
23	A	A	A	F	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MSH2
26	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
27	E	E	E	F	E	F	E	E	E	E	F	E	E	E	E	E	E	U	E	E	E	E	E	E	MLH1
32	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	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	MLH1
35	E	E	F	F	F	F	E	U	F	E	F	E	U	U	U	U	U	U	U	U	E	U	E	U	MSH6
38	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	MSH2

MSH6: MSH6 staining results were excellent. Core 3 had variable and weak MSH6 weak staining that can be seen with MSH2 loss**.** Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
102	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
125	Optimal	
138	Optimal	
141	Optimal	Staining artifact appears clustered around Cores 21-26; uninterpretable
144	Optimal	
149	Optimal	

Lab ID	IHC Status*	Comments
175	Optimal	Slightly weak
181	Optimal	
186	Adequate	General background staining
190	Optimal	
194	--	Slide not available
202	Optimal	
207	Optimal	
217	Optimal	Slight background blush in some cores
220	Optimal	
222	Optimal	Primary Ab may not have been applied to several cores closest to the slide label; staining on the rest of the slide is technically optimal
231	Optimal	

*based on cIQc assessment

Garrattogram after cIQc assessment of MSH6 (with unsatisfactory cores removed if present in more than 50% of labs):

Lab/ Core	102	106	107	109	110	111	112	114	125	138	141	144	149	175	181	186	190	194	202	207	217	220	222	231	MMR Status
1	E	U	E	E	E	U	U	U	E	E	U	U	U	U	U	E	U	U	E	E	E	E	F	E	MLH1
2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	MSH6
3	A	A	A	A	A	A	A	A	U	A	A	E	A	A	U	F	A	U	U	U	U	U	F	U	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	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	PMS2
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	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	U	E	E	U	E	U	MLH1
8	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	U	A	MLH1
11	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
12	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2
14	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	MSH6
15	A	A	A	F	A	A	F	F	A	A	A	F	A	F	F	A	F	A	A	A	A	F	F	F	MSH6
16	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
18	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	MSH6
19	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	MLH1
20	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
21	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	F	E	MLH1
22	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	F	E	MLH1
23	A	A	A	A	A	A	A	A	A	A	U	A	A	F	A	A	A	A	A	A	A	A	F	A	MSH2
26	E	E	E	E	E	E	E	E	E	E	U	F	E	E	E	E	E	E	E	E	E	E	F	E	MLH1
27	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	F	A	MLH1
32	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	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	MLH1
35	A	A	A	F	A	A	F	U	A	F	A	F	U	U	U	U	U	U	U	U	A	U	F	U	MSH6
37	E	E	E	E	E	E	E	E	E	U	U	E	U	U	U	U	U	U	U	U	U	U	U	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	A	MSH2

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
102	DAKO PT - HIGH PH	20	ES05	1:80	Dako	10117650	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
106	Microwave pressure coooker	30	ESO5	1:100	Leica	6050125	45	MACH4	no	no	DAB
107	Ultra cc1	64	ES05	1:20	Leica/Novocastra	6050125	40	Optiview DAB	N	Y	DAB
109	HIER high pH CC1	32 MIN	M1	RTU	ROCHE	V17376	16 MIN	OPTIVIEW	N	Y	DAB
110	High pH	20	ES05	1:100	DAKO	10127457	20	Envision Flex	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	ESO5	1:100	Leica (Novocastra)	6042101	30 minutes	BOND polymer refine	none	none	DAB
114	High PH	30	ES05	RTU	DAKO	10125751	20	Envision FLEX DAKO Omnis	Y	N	DAB
138	HIER (high pH)	20	ES05	RTU	Dako	10128478	20	Dako Flex Envision	Y	N	DAB
141	HIER	20	ES05	1:100	DAKO	10121400	20	POLYMER	Y	N	DAB
144	U. CC1	56 min	ES05	1:25	Novocastra	6046423	32 min.	OptiView	No	Yes, copper	DAB
149	PT Link high pH	20 min at 97 C	ES05	RTU	Dako Agilent	1013475	30	EnVision Flex	No	No	DAB
175	HIER	64	M1	Pre-dilute	Roche	Y10956	16	polymer	N	Y	DAB
181	hier pH10	20	ES05	1:100	DAKO	10127457	20	HRP POLYMER	Y	N	DAB
186	HIER	20	G168-728	1:50	CELL MARQUE	1614602A	15	BOND REFINE DETECTION KIT	N	N	DAB
190	CC1	40	ES05	1:50	DAKO	10127457	40	OPTIVIEW DAB	Y	Y	N
194	CC1	64	M1	RTU	VENTANA-ROCHE	Y17376	16	OPTIVIEW	N	N	DAB
202	ER2	20	ES05	1/10	BD Pharmagen	61402540	16	Refine Detection system	no	no	DAB
207	Envision Flex High PH	30 minutes	ES05	RTU	Dako	10130475	25 minutes	Envision Flex DAB	Y	Y	DAB
217	HIER CC1	64	M1	RTU	Roche Ventana	Y06056Z	60	Optiview	N	Y	DAB
220	CC1	72	ES05	1/25	DAKO	10128797	1hr	OptiView	N	N	DAB
222	Ultra CC1	40	ES05	1:1	Ventana	G09887	4	Optiview DAB	Y	Y	Copper
231	HIER	56 mins	M1	PRE-DILUTE	ROCHE/VENTANA	G05543	1 hr	OPTIVIEW	N	Y	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
102	DAKO PT - HIGH PH	20	EP51	1:20	Dako	10132271	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
106	micrwave pressure cooker	30	MRQ-28	1:25	Cell Marque	10240	45	MACH4	no	no	DAB
107	Decloak FLEX TRS High pH	120celcius, 30seconds	A16-4	1:200	BD Pharmingen	7103847	30	FLEX 30+	N	N	DAB
109	HIER high pH cc1	64 min	EPR3947	RTU	ROCHE	V0000985	20 MIN	OPTIVIEW	Y	Y	DAB
110	High pH	20 min	EP51	1:50	DAKO	10126823	30 min	Dako Envision Flex	N	N	DAB
111	HIER	48	EP51	1/100	DAKO	10132271	32	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	30 minutes	EP51	1:75 using DAKO background reducing diluent	DAKO	10132224	30 minutes	BOND polymer refine	none	none	DAB
114	High PH	30	EP51	RTU	DAKO	10127412	20	Envision FLEX DAKO Omnis	Y	N	DAB
138	HIER (High pH)	20	EP51	RTU	Dako	10129191	30	Dako Flex Envision	Y	N	DAB
141	HIER	20	EP51	1:50	DAKO	10126823	30 min	POLYMER	Y	N	DAB
144	U. CC1	64 min	EPR3947	Pre-Dilute	Cell Marque	V985	60 min.	OptiView	No	Yes, copper	DAB
149	PT Link high pH	20 min at 97 C	EP51	RTU	Dako Agilent	10130585	20	EnVision Flex	Yes	No	DAB
175	HIER	64	EPR3947	Pre-dilute	Roche	V0000327	32	Polymer	Y	Y	DAB
181	HIER PH10	20	EP51	1:50	DAKO	10126823	30	HRP POLYMER	N	N	DAB
186	HIER	20	ERP3947	1:4	CELL MARQUE	20343	15	BOND REFINE DETECTION KIT	N	N	DAB
190	CC1	48	EPR3947	Predilute	Cell Marque	1626504E	32	OPTIVIEW DAB	Y	N	N
194	CC1	64	EPR3497	RTU	CELL MARQUE (ROCHE)	V0000985	32	OPTIVIEW	N	N	DAB
202	ER2	30min	a16-4	1/25	BD Pharmagen	7103847	16	Refine Detection system	no	no	dab
207	Envision Flex High PH	30 minutes	EP51	RTU	DAKO	10131396	20 minutes	Envision Flex DAB	Y	Y	DAB
217	HIER CC1	40	EPR3947	RTU	Roche Ventana	V0000327	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	V0000780	32	Optiview DAB	Y	Y	Copper
231	HIER	64 mins	EP51	1/25	DAKO	10132271	1 HR 20 MINS	ULTRAVIEW	Y	Y	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
102	DAKO PT - HIGH PH	20	FE11	1:40	Dako	10125046	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
106	microwave pressure cooker	30	FE11	1:125	Dako	10128612	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	V0000777	8 MIN	OPTIVIEW	N	Y	DAB
110	High pH	20 min	FE11	1:300	DAKO	10126581	20 min	Dako Envision Flex	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	1:00	DAKO	10128612	30 minutes	BOND polymer refine	none	none	DAB
114	High PH	30	FE11	RTU	DAKO	10127888	30	Envision FLEX DAKO Omnis	N	N	DAB
138	HIER (high pH)	20	FE11	RTU	Dako	10131394	20	Dako Envision Flex	Y	N	DAB
141	HIER	20	FE11	1:300	DAKO	10126581	20 min	POLYMER	Y	N	DAB
144	U. CC1	40 min	G219-1129	Pre-Dilute	Cell Marque	1529502G	16 min.	OptiView	No	Yes, copper	DAB
149	PT Link high pH	20 min at 97 C	FE11	RTU	Dako Agilent	10129810	20	EnVision Flex	Yes	No	DAB
175	HIER	32	g219-1129	Pre-dilute	Roche	V0000777	16	Polymer	N	Y	DAB
181	HIER PH10	20	FE11	1:300	DAKO	10126581	20	HRP POLYMER	Y	N	DAB
186	HIER	20	G219-1129	1:200	CELL MARQUE	1620915E	15	BOND REFINED DETECTION KIT	N	N	DAB
190	CC1	32	G219-1129	1/100	Cell Marque	5920	32	OPTIVIEW DAB	N	N	N
194	CC1	32	G219-1129	RTU	CELL MARQUE (ROCHE)	V0000775	16	OPTIVIEW	N	N	DAB
202	ER2	20	G219-1129	1/100	Cell marque	20622	16	Refine Detection system	no	no	DAB
207	Envision Flex High PH	30 minutes	FE11	RTU	DAKO	10131394	30	Envision Flex DAB	N	Y	DAB
217	HIER CC1	56	G219-1129	RTU	Roche Ventana	V0000154	32	Optiview	N	Y	DAB
220	CC1	32min	G219-1129	Pre-Dilute	Cell Marque	V0000775	32min	OptiView	N	N	DAB
222	Ultra CC1	64	G219-1129	1:1	Ventana	V000075	24	Optiview DAB	Y	Y	Copper
231	HIER	64 mins	G219-1129	PRE-DILUTE	ROCHE/VENTANA	1529502 C	20 mins	ULTRAVIEW	Y	Y	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
102	DAKO PT - HIGH PH	20	EP49	1:100	Dako	10116602	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
106	Mocrwave pressure cooker	20	SP93	1:80	Cell Marque	9803	45	MACH4	no	no	DAB
107	Ultra cc1	56	EPR 3945	1:750	Abcam	GR262215-19	32	Optiview DAB	N	Y	DAB
109	HIER high pH CC1	64 MIN	44	RTU	ROCHE	Y18878	20 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	EP49	1:200	DAKO	10125054	30 min	Dako Envision Flex	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	Epitomics/Cell Marque	EN072101	30 minutes	BOND polymer refine	none	none	DAB
114	High PH	30	EP49	RTU	DAKO	10128249	20	Envision FLEX DAKO Omnis	N	n	DAB
138	HIER (high pH)	20	EP49	RTU	Dako	10123749	20	Dako Envision Flex	Y	N	DAB
141	HIER	20	EP49	1:200	DAKO	10125054	30	POLYMER	y	n	DAB
144	U. CC1	32 min	EP49	1:25	Dako	10128801	32 min.	OptiView	No	Yes, copper	DAB
149	PT Link high pH	20 min at 97 C	EP49	RTU	Dako Agilent	10123749	20	EnVision Flex	No	No	DAB
175	HIER	64	44	1 in 100	Cell Marquw	1510722B	24	Polymer	Y	Y	DAB
181	HIER PH10	20	EP49	1:200	DAKO	10125054	30	HRP POLYMER	N	N	DAB
186	HIER	20	BC/44	1:100	BIOCARE	51816	15	BOND REFINE DETECTION KIT	N	N	DAB
190	CC1	32	EP49	1/100	Cedarlane/Epitomics	EN020910	32	OPTIVIEW DAB	N	N	N
194	CC1	63	44	RTU	VENTANA-ROCHE	Y23047	16	OPTIVIEW	N	N	DAB
202	ER2	40	EPR3945	1/25	ABCAM	GR262215	16	Refine Detection system	no	no	DAB
207	Envision Flex High PH	30 minutes	EP49	RTU	DAKO	10133546	20 minutes	Envision Flex DAB	N	Y	DAB
217	HIER CC1	64	GTBP45	1:3000	Roche Ventana	Y09211	60	Optiview	N	Y	DAB
220	CC1	32min	EP49	1/50	DAKO	10114097	28min	OptiView	N	N	DAB
222	Ultra CC1	56	44	1:1	Ventana	Y15678	16	Optiview DAB	Y	Y	Copper
231	HIER	64 mins	EP49	1/25	DAKO	10128801	32 mins	ULTRAVIEW	Y	Y	DAB

Table S5. Descriptive statistics for MLH1 based on cIQc assessment (cores 1, 25, 29, 30 and 39 were excluded due to patchy expression or unsatisfactory scores for more than 50% of labs).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
102	35	77.14	27	26/27 (96%)	0.93	1	0.93
106	35	77.14	27	26/27 (96%)	0.93	1	0.93
107	35	88.57	31	29/31 (94%)	0.94	0.93	0.87
109	35	82.86	29	28/29 (97%)	0.93	1	0.93
110	35	91.43	32	30/32 (94%)	0.94	0.94	0.88
111	35	91.43	32	30/32 (94%)	0.94	0.94	0.88
112	35	77.14	27	26/27 (96%)	0.93	1	0.93
114	35	62.86	22	21/22 (95%)	0.92	1	0.91
125	35	77.14	27	26/27 (96%)	0.93	1	0.93
138	35	74.29	26	25/26 (96%)	0.93	1	0.92
141	35	77.14	27	26/27 (96%)	0.93	1	0.93
144	35	74.29	26	24/26 (92%)	0.92	0.92	0.85
149	35	77.14	27	26/27 (96%)	0.92	1	0.93
175	35	77.14	27	26/27 (96%)	0.93	1	0.93
181	35	68.57	24	23/24 (96%)	0.92	1	0.92
186	35	80	28	26/28 (93%)	0.92	0.93	0.86
190	35	74.29	26	25/26 (96%)	0.92	1	0.92
194	35	60	21	21/21 (100%)	1	1	1
202	35	71.43	25	24/25 (96%)	0.92	1	0.92
207	35	68.57	24	23/24 (96%)	0.92	1	0.92
217	35	74.29	26	25/26 (96%)	0.92	1	0.92
220	35	68.57	24	23/24 (96%)	0.92	1	0.92
222	35	77.14	27	26/27 (96%)	0.93	1	0.93
231	35	71.43	25	24/25 (96%)	0.92	1	0.92

Table S6. Descriptive statistics for PMS2 based on cIQc assessment (cores 9, 36, and 37 were excluded due to patchy expression).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
102	37	83.78	31	31/31 (100%)	1	1	1
106	37	75.68	28	28/28 (100%)	1	1	1
107	37	89.19	32	32/32 (100%)	1	1	1
109	37	75.68	28	28/28 (100%)	1	1	1
110	37	94.59	34	34/34 (100%)	1	1	1
111	37	70.27	26	26/26 (100%)	1	1	1
112	37	72.97	27	27/27 (100%)	1	1	1
114	37	56.76	21	21/21 (100%)	1	1	1
125	37	70.27	26	26/26 (100%)	1	1	1
138	37	75.68	28	28/28 (100%)	1	1	1
141	37	78.38	28	28/28 (100%)	1	1	1
144	37	70.27	26	26/26 (100%)	1	1	1
149	37	72.97	27	27/27 (100%)	1	1	1
175	37	67.57	25	25/25 (100%)	1	1	1
181	37	64.86	24	24/24 (100%)	1	1	1
186	37	75.68	28	28/28 (100%)	1	1	1
190	37	64.86	24	23/24 (96%)	1	0.93	0.91
194	37	59.46	22	22/22 (100%)	1	1	1
202	37	64.86	24	24/24 (100%)	1	1	1
207	37	64.86	24	24/24 (100%)	1	1	1
217	37	78.38	28	28/28 (100%)	1	1	1
220	37	67.57	25	25/25 (100%)	1	1	1
222	37	72.97	27	27/27 (100%)	1	1	1
231	37	51.35	19	19/19 (100%)	1	1	1

Table S7. Descriptive statistics for MSH2 based on cIQc assessment (core 36 was excluded from analysis due to very few tumour cells).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
102	39	84.62	33	33/33 (100%)	1	1	1
106	39	74.36	29	29/29 (100%)	1	1	1
107	39	89.74	35	35/35 (100%)	1	1	1
109	39	66.67	26	26/26 (100%)	1	1	1
110	39	89.74	35	35/35 (100%)	1	1	1
111	39	74.36	29	29/29 (100%)	1	1	1
112	39	71.79	28	28/28 (100%)	1	1	1
114	39	61.54	24	24/24 (100%)	1	1	1
125	39	69.23	27	27/27 (100%)	1	1	1
138	39	79.49	31	31/31 (100%)	1	1	1
141	39	64.1	25	25/25 (100%)	1	1	1
144	39	71.79	28	28/28 (100%)	1	1	1
149	39	74.36	29	29/29 (100%)	1	1	1
175	39	61.54	24	24/24 (100%)	1	1	1
181	39	66.67	26	26/26 (100%)	1	1	1
186	39	71.79	28	28/28 (100%)	1	1	1
190	39	64.1	25	25/25 (100%)	1	1	1
194	39	48.72	19	18/19 (95%)	0.94	1	0.83
202	39	66.67	26	26/26 (100%)	1	1	1
207	39	66.67	26	26/26 (100%)	1	1	1
217	39	82.05	32	32/32 (100%)	1	1	1
220	39	64.1	25	25/25 (100%)	1	1	1
222	39	69.23	27	27/27 (100%)	1	1	1
231	39	58.97	23	23/23 (100%)	1	1	1

Table S8. Descriptive statistics for MSH6 based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
102	40	80	32	32/32 (100%)	1	1	1
106	40	72.5	29	29/29 (100%)	1	1	1
107	40	95	38	37/38 (97%)	0.96	1	0.94
109	40	75	30	29/30 (97%)	0.95	1	0.93
110	40	87.5	35	34/35 (97%)	0.96	1	0.94
111	40	82.5	33	32/33 (97%)	0.95	1	0.94
112	40	72.5	29	29/29 (100%)	1	1	1
114	40	62.5	25	25/25 (100%)	1	1	1
125	40	75	30	30/30 (100%)	1	1	1
138	40	72.5	29	29/29 (100%)	1	1	1
141	40	57.5	23	23/23 (100%)	1	1	1
144	40	60	24	23/24 (96%)	1	0.89	0.91
149	40	70	28	28/28 (100%)	1	1	1
175	40	57.5	23	23/23 (100%)	1	1	1
181	40	60	24	24/24 (100%)	1	1	1
186	40	67.5	27	27/27 (100%)	1	1	1
190	40	65	26	26/26 (100%)	1	1	1
194	40	52.5	21	21/21 (100%)	1	1	1
202	40	62.5	25	25/25 (100%)	1	1	1
207	40	65	26	26/26 (100%)	1	1	1
217	40	77.5	31	31/31 (100%)	1	1	1
220	40	62.5	25	25/25 (100%)	1	1	1
222	40	42.5	17	17/17 (100%)	1	1	1
231	40	62.5	25	25/25 (100%)	1	1	1