Assessors' report for cIQc Run 70: MMR Immunohistochemistry

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Assessment performed on Tuesday, June 6th, 2017 at St. Paul's Hospital, Vancouver, BC

Overview

MMR immunohistochemical staining continues to be of good quality. Slides submitted in time for the assessment meeting were blindly reviewed by cIQc assessors. Independent review led to alteration of original self-reported results for discordant cores. With regards to interpretation of MMR staining, it is important to ensure that there is appropriate internal control staining before diagnosis of loss of expression. MMR proteins are very fixation sensitive and degrade rapidly. Unfortunately, significant core dropout or exhaustion of tumour within certain tissue microarray cores was a problem for this cIQc challenge. For these reasons, Cores 3, 13, 14, 17, 24, 25, 28, 30, 31, 35, 36, 37, 39 and 40 were excluded from all analyses for all MMR proteins in this report.

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. Please note that absent staining is not always indicative of an underlying germline 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.

As noted in previous reports, tumours with methylation of the promoter of MLH1 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). Furthermore, an increased rate of "failed" tests 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.

MLH1

MLH1 staining results were, overall, very good. While variability in staining intensity is expected of immunohistochemistry, weaker staining by some labs (relative to other labs) was observed. Labs 114 and 124 had particularly weak staining that made interpretation challenging or not possible for some cores (i.e. failed results) that were easily interpretable on slides from other labs. Participant specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Adequate	Weak staining
102	Optimal	
103	Optimal	
104	Optimal	Nice staining
106	Optimal	Nice staining
107	Optimal	
109	Optimal	
110	Optimal	Nice staining
111	Optimal	Nice staining
112	Optimal	Nice staining
114	Sub-optimal	Faint stromal (control) staining (e.g. Core 15)
115	Optimal	
116	Optimal	

Lab ID	IHC Status*	Comments
123	Adequate	Slightly weak staining
124	Failed	Weak staining leading to several failed cores; assay has poor sensitivity
125	Optimal	
126	Optimal	
138	Optimal	
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Adequate	Slightly weak staining
186	Adequate	Slightly weak staining
189	Optimal	
202	Adequate	Slightly weak staining
207	Optimal	
217	Optimal	
220	Optimal	
222	Optimal	
231	Adequate	Weak staining

*Based on cIQc assessor concensus

Lab/ Core	101	102	103	104	106	107	109	110	111	112	114	115	116	123	124	125	126	138	141	144	145	149	175	181	186	189	202	207	217	220	222	231	**MMR status**
1	A	A	A	A	A	A	A	A	A	A	A	U	U	U	U	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	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	E	MSH6
4	A	U	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	U	MLH1
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2
6	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	E	E	E	PMS2
7	U	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	A	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	E	MSH2
9	A	A	A	A	A	A	U	A	U	A	U	U	U	A	U	A	U	U	A	A	U	U	A	A	U	A	A	A	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	A	A	A	A	U	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	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	E	E	E	E	E	E	E	E	PMS2
15	E	E	E	E	E	E	E	E	E	E	F	E	E	E	F	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E	MSH6
16	A	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
18	U	U	U	U	U	U	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	MSH6
19	E	U	U	U	A	A	U	A	A	U	A	A	A	A	A	A	A	U	U	U	A	A	U	U	A	U	U	A	A	A	U	A	MLH1
20	U	U	U	U	U	U	U	A	A	A	A	A	A	A	A	U	A	U	A	U	A	A	U	A	A	U	U	A	U	A	U	A	MLH1
21	A	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	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	A	A	A	A	A	A	A	A	MLH1
23	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	E	E	MSH2
26	A	A	A	A	A	A	A	A	A	A	A	U	U	U	F	A	U	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	F	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1/MSH6
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	A	A	A	A	A	A	A	A	MLH1
33	A	A	U	A	A	A	A	A	A	A	A	U	U	U	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	A	A	A	F	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

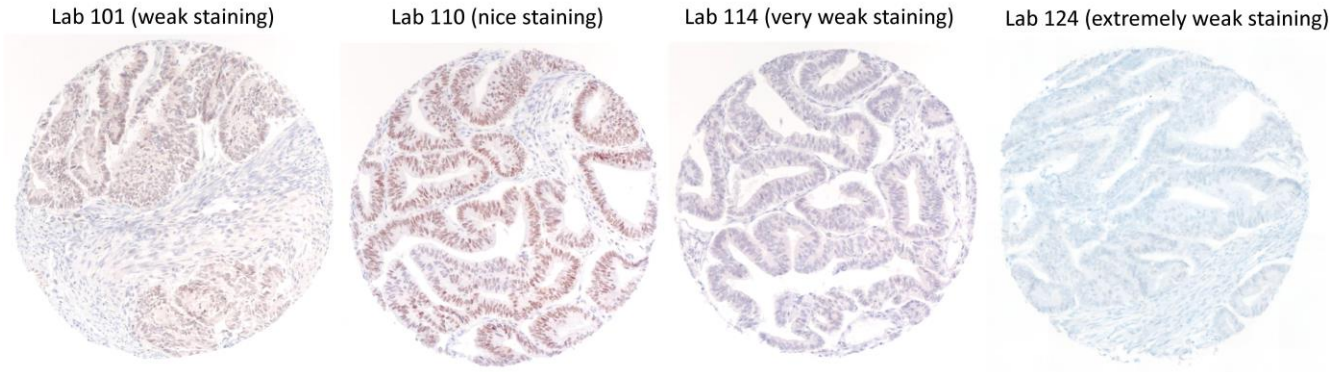

Figure 1. Representative staining of Core 15, a case expressing MLH1, by different labs.

PMS2: PMS2 staining results were also good, with only two sub-optimal results due to either very weak staining or excessive background. Participant specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	
103	Adequate	Background staining
104	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
110	Adequate	Slightly weak staining
111	Optimal	
112	Adequate	Slightly weak staining
114	Optimal	
115	Adequate	High background staining
116	Adequate	Slightly weak staining
123	Optimal	
124	Adequate	Weak staining
125	Optimal	
126	Optimal	
138	Optimal	
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	Slightly weak staining
202	Optimal	
207	Sub-optimal	Very weak staining
217	Sub-optimal	Excessive background staining
220	Optimal	
222	Adequate	High background staining (consistent with self-assessor's comments); glad to know the protocol has already been re-optimized to reduce background
231	Adequate	Weak staining

*Based on cIQc assessor concensus

Lab/ Core	101	102	103	104	106	107	109	110	111	112	114	115	116	123	124	125	126	138	141	144	145	149	175	181	186	202	207	217	220	222	231	**MMR status**
1	A	A	A	A	A	A	A	A	A	A	A	U	U	U	U	A	A	U	U	A	A	A	A	A	A	A	A	A	A	A	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
4	A	U	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	U	MLH1
5	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	PMS2
6	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	E	A	A	A	A	PMS2
7	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	A	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	A	A	U	A	A	U	U	A	U	A	U	U	U	U	U	A	A	U	U	A	A	U	U	U	A	A	A	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	A	A	A	U	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	E	E	E	E	E	E	U	MSH2
12	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	E	A	A	A	A	PMS2
15	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	F	MSH6
16	A	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
18	U	U	U	E	U	U	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	MSH6
19	U	U	U	U	A	A	U	A	A	U	A	A	A	U	A	A	U	A	A	U	U	A	U	A	A	U	U	A	A	U	A	MLH1
20	U	U	U	U	U	U	U	A	A	A	A	A	A	A	A	U	U	A	A	A	U	A	U	A	A	A	A	A	A	A	A	MLH1
21	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	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	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	E	E	E	E	E	E	F	MSH2
26	A	A	A	A	A	A	A	A	A	A	A	U	U	U	A	A	A	U	U	A	A	A	A	A	A	A	A	A	A	A	A	MLH1
27	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	F	MLH1/MSH6
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	A	A	A	A	A	A	A	MLH1
33	A	A	F	A	A	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	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	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	MSH2

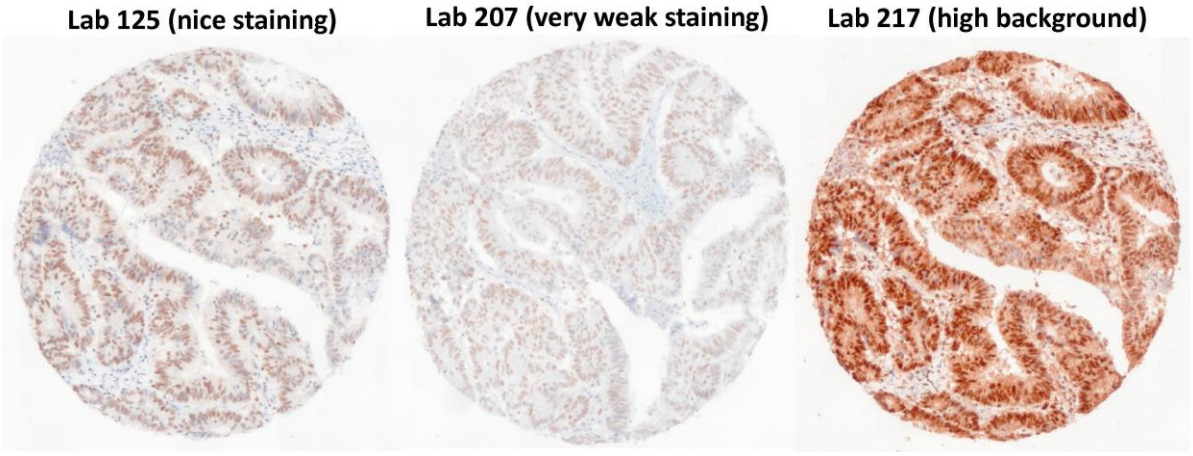

Figure 2. Representative staining of Core 8, a case expressing PMS2, by different labs.

MSH2: MSH2 staining results were excellent. Weaker staining or slight background staining were noted in some labs, relative to other labs, but results were interpretable. Participant specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Adeuqate	Slightly weak staining
102	Optimal	
103	Optimal	
104	Optimal	
106	Optimal	
107	Adeuqate	Weak staining particularly in weak expressing cores (e.g. Core 15 and 27)
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
115	Optimal	
116	Optimal	
123	Adeuqate	Slight background
124	Optimal	
125	Optimal	
126	Optimal	
138	Optimal	Particularly nice staining
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	Particularly nice staining
186	Adeuqate	Slightly weak staining
189	Optimal	
202	Optimal	Technical issue seems to have occurred on the last 3 columns of the tissue microarray
207	Optimal	
217	Optimal	Very slight background
220	Optimal	
222	Optimal	
231	Optimal	

*Based on cIQc assessor concensus

Lab/ Core	101	102	103	104	106	107	109	110	111	112	114	115	116	123	124	125	126	138	141	144	145	149	175	181	186	189	202	207	217	220	222	231	**MMR status**
1	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E	E	E	E	E	E	E	U	E	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	E	E	E	E	E	E	E	E	MSH6
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	E	E	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	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	E	E	E	E	E	E	E	PMS2
7	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	A	A	A	A	A	A	A	A	MSH2
9	E	E	E	E	E	E	U	E	U	E	U	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	U	E	E	MLH1
10	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
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	A	A	F	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	E	E	F	E	E	E	E	E	PMS2
15	F	E	E	E	E	F	E	E	F	E	F	E	F	F	F	E	E	E	E	E	F	E	E	E	F	E	A	A	F	F	F	F	MSH6
16	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
18	U	U	U	U	E	U	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	MSH6
19	E	E	E	E	E	U	U	E	E	U	E	E	E	E	E	E	U	E	E	E	U	E	U	U	E	E	E	U	E	E	E	E	MLH1
20	U	U	U	U	U	U	U	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	U	E	E	U	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	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	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	E	A	A	A	A	A	MSH2
26	E	E	E	E	E	E	E	E	E	E	E	U	U	U	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
27	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	E	F	E	E	E	E	E	MLH1/MSH6
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	E	E	E	E	E	E	E	E	MLH1
33	E	E	E	E	E	E	E	E	E	E	E	U	U	U	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	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	A	A	A	F	A	A	A	A	A	MSH2

MSH6: MSH6 staining has gradually improved compared to previous challenges and results were good, overall. Only one sub-optimal result due to very weak staining (Lab 144) was observed. Cores 2 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. Participant specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	
103	Optimal	
104	Optimal	
106	Optimal	
107	Adequate	Weak control (i.e. stromal) staining
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
115	Optimal	
116	Optimal	
123		
124	Optimal	
125	Optimal	
126	Optimal	
138	Optimal	
141	Optimal	
144	Sub-optimal	Very weak staining
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	High cytoplasmic background staining
189	Optimal	
202	Optimal	Technical issue seems to have occurred on the last 3 columns of the tissue microarray
207	Optimal	
217	Adequate	Slight background
220	Optimal	
222	Optimal	
231	Optimal	

*Based on cIQc assessor concensus

Lab/ Core	101	102	103	104	106	107	109	110	111	112	114	115	116	123	124	125	126	138	141	144	145	149	175	181	186	189	202	207	217	220	222	231	**MMR status**
1	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	U	E	E	E	E	E	E	E	U	E	E	E	E	U	MLH1
2	A	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	A	A	A	A	A	MSH6
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	E	E	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	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	E	E	E	E	E	E	E	PMS2
7	U	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	E	E	E	E	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	A	A	A	A	A	A	A	A	MSH2
9	E	E	E	E	E	E	U	E	U	E	E	E	E	E	E	E	E	U	E	E	E	U	U	E	E	E	E	E	U	U	E	E	MLH1
10	E	E	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	A	MLH1
11	A	E	A	A	A	A	A	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	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	E	PMS2
15	F	F	F	A	A	A	F	A	A	F	F	A	A	F	F	A	A	F	A	A	A	A	A	F	A	A	A	F	A	F	F	F	MSH6
16	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
18	U	U	U	U	U	U	U	A	A	U	A	A	A	A	A	A	A	A	A	F	A	A	A	U	A	U	A	F	A	A	A	A	MSH6
19	E	E	U	E	E	U	U	E	E	U	E	E	U	E	E	U	U	E	E	U	U	E	U	U	U	E	E	U	E	E	E	E	MLH1
20	U	U	U	U	U	U	U	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	U	E	U	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	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	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	E	A	A	A	A	A	MSH2
26	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
27	U	A	A	A	A	A	E	A	A	A	A	A	A	A	A	A	A	A	A	F	E	A	A	E	A	A	A	F	A	A	A	E	MSH6
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	E	E	E	E	E	E	E	E	MLH1
33	E	E	E	E	E	E	E	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	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	E	E	MLH1
38	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	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
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	DAB+
103	CC1	56 MINS	M1	PRE	vENTANA	F02382	48 MINS	OPTIVIEW	N	COPPER	DAB
104	HIER	20	ES05	RTU	DAKO	10107799	30 min	polymere	y	n	DAB
106	microwave pressure cooker	30	ES05	1:105	Novacastra	44922	45	MACH4	no	no	DAB
107	ultra cc1	64	E505	1:20	Leica/Novocastra	6043892	40 min	Optiview DAB	N	Y	DAB
109	HIER high pH	32 MIN	M1	RTU	VENTANA	F10114	16 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High ph 9.0	20 min @97C	ES05	1:100	DAKO	10107954	20 min	Dako Envision Flex	Y	N	DAB
111	HIER CC1	48 min	G168-15	1/50	Biocare	81716	32 min	optiview	Y	Y	DAB
112	BOND Epitope Retrieval 2	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	Copper	DAB
115	Envision Flex High pH	30 mins	ESO5	RTU	Dako	10117334	20 mins	Envision Flex	N	N	DAB
116	CC1	48 MIN	G168-15	1/80	BD PHARMINGEN	5329901	44 MIN	OPTIVIEW DAB	Y	.	DAB
124	CC1	64	MUM1p	1/100	Leica	6043892	16	Opti	O	n	dab
126	Microwave pressure cooker with Citrate buff pH 6.01	36	ES05	1:100	Dako	10117650	30	Quanto polymer	no	no	DAB+
138	High pH HIET	20	ES05	RTU	Dako	10117334	20	Dako Envision Flex	Y	N	DAB
141	HIER	20	ES05	1:100	Dako	10107954	20	Polymer	Y	N	DAB
144	CC1	56 min	E505	1:25	Novocastra	6039695	32 min.	OptiView	No	Copper	DAB
145	CC1	56	G168-15	1/50	BIOCARE	20215	32	VENTANA XT OPTIVIEW ihc v4	N	N	DAB
149	PT Link high pH	20 min at 97 C	ES05	RTU	Dako	10115068	30	EnVision Flex	Yes	No	DAB
175	HIER	64	M1	Pre -dilute	ROche	F10114	16	Polymer	N	Y	DAB
181	HIER	20	ES05	1:100	DAKO	10107954	20	HRP-polymer	N	N	DAB
186	HIER	20	G168-728	1:50	Cell Marque	1313506H	15	LEICA BOND	N	N	DAB
189	CC1	64	M1	Pre-dilute	Ventana	unknown	24	OptiView DAB	N	N	OptiView DAB
202	HIER citrate pH 9.5	20	ES05	1/10	BDPHARMAGEN	6140540	15	Leica Refine detection kit	no	no	DAB
207	on line-CC1	32 minutes	M1	Prediluted	ventana	G02350	20 miutes	Ultraview DAB	N	Y	DAB
220	CC1	72min	ES05	1/25	DAKO	10114422	1hr	OptiView	N	N	DAB
222	CC1	40	M1	RTU	Ventana	G05543	4	Optiview DAB	Y	Y	Copper
231	CC1	64 mins	M1	RTU	ROCHE/VENTANA	G03827	40 mins	ULTRAVIEW (VENTANA)	Y	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
101	CC1	32 min	EP51	1:15	Epitomics	EN051809	32min	Optiview	N	N	DAB
102	DAKO PT - HIGH PH	20	EP51	1:20	DAKO	10117823	30" RT	DAKO ENVISION FLEX+	YES	YES	DAB+
103	CC1	64 MINS	EPR3947	PRE	CELL MARQUE	1506813B	1 HOUR	OPTIVIEW	YES	COPPER	DAB
104	HIER	20 min	EP51	RTU	DAKO	10114394	20 min	polymere	y	n	DAB
106	microwave pressure cooker	30	MRQ-28	1:25	Cell Marque	1516709B	45	MACH4	no	no	DAB
107	Decloaking in Flex TRS High p49-0	120C 30sec	A16-4	1:200	BD Pharmingen	6300808	30 min	Flex 30+	N	N	DAB
109	HIER high pH	64 MIN	EPR3947	RTU	CELL MARQUE	1607510B	20 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0	20 min @97C	EP51	1:50	DAKO	10117823	30 min	Dako Envision Flex	N	N	DAB
111	HIER CC1	48 min	EP51	1/100	DAKO	10112572	32 min	Optiview	Y	Y	DAB
112	BOND Epitope Retrieval 2	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	EN041303	64	Optiview	N	Copper	DAB
115	Envision Flex High pH	30 mins	EP51	RTU	Dako	10111642	30 mins	Envision Flex	N	N	DAB
116	CC1	64 min	EPR3947	RTU	VENTANA	1607510 C	60MIN	OPTIVIEW DAB	Y	.	DAB
124	CC1	56	EP51	1/40	Dako	10118334	32	Opti-view	n	n	DAB
126	Microwave pressure cooker, with Citrate buffer pH 6.01	36	EPS1	1:25	Dako	10121526	30	Quanto polymer	no	no	DAB+
138	High pH HIET	20	EP51	RTU	Dako	10111642	30	Dako Envision Flex	N	N	DAB
141	HIER	20	EP51	1:50	DAKO	10117823	30	Polymer	Y	N	DAB
144	CC1	64 min	EPR3947	Pre-Dilute	Cell Marque	1626504C	60 min	OptiView	No	Copper	DAB
145	CC1	56	EPR3947	pre-diluted	CELLMARQUE	1506813A	24	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	EP51	RTU	Dako	10114394	20	EnVision Flex	Yes	No	DAB
175	HIER	64	EPR3947	Predilute	Roche	1607510C	32	polymer	Y	Y	DAB
181	HIER	20	EP51	1:50	DAKO	10117823	30	HRP-polymer	N	N	DAB
186	HIER	20	ERP3947	1:4	Cell Marque	1506813F	15	LEICA BOND	N	N	DAB
202	HIER citrate pH 9.5	30 min	a16-4	1/25	BDPHARMAGEN	6300808	15 MIN	Leica Refine detection kit	no	no	DAB
207	on line CC1	64 miutes	EPR3347	Prediluted	Cell Marque	1532405 F	8	Ultraview DAB	N	Y	DAB
220	CC1	64min	EP51	1/40	DAKO	10112572	1h8min	OptiView	N	N	DAB
222	CC1	92	EPR3947	RTU	Ventana	1626504B	28	Optiview DAB	Y	Y	Copper
231	CC1	64 mins	EP51	1/25	DAKO	10117823	80 mins	ULTRAVIEW (VENTANA)	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
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	10117989	30" RT	DAKO ENVISION FLEX	YES	YES	DAB+
103	CC1	56 MINS	G219-112 9	PRE	CELL MARQUE	1417104F	32	OPTIVIEW	N	COPPER	DAB
104	HIER	20 min	FE11	RTU	DAKO	10120750	30 min	polymere	y	n	DAB
106	microwave pressure cooker	30	FE11	1:30	Dako	10117989	45	MACH4	no	no	DAB
107	cc1	32	G219 - 1129	1:200	Cell Marque	1505809A	32 min	Optiview DAB	N	Y	DAB
109	HIER high pH	32 MIN	G221-1129	RTU	CELL MARQUE	1529502F	8 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High ph 9.0	20 min @97C	FE11	1:150	DAKO	10106449	20 min	Dako Envision Flex	Y	N	DAB
111	HIER CC1	40 min	G219-1129	1/600	Cello Marque	1616010a	32 min	Optiview	Y	Y	DAB
112	BOND Epitope Retrieval 2	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	Copper	DAB
115	Envision Flex High pH	30 mins	FE11	RTU	DAKO	10112557	20 mins	Envision Flex	N	N	DAB
116	CC1	40 MIN	G219-1129	1/400	CELL MARQUE	1505809 F	48 MIN	OPTIVIEW DAB	N	.	DAB
124	CC1	24	G219-1129	PrÃ©-diluÃ©	Cell Marque	1529502F	20	Opti	n	n	dab
126	Microwave pressure cooker with Citrate buffer pH 6.01	36	FE11	1:75	Dako	10117989	30	Quanto polymer	no	no	DAB+
138	High pH HIET	20	FE11	RTU	Dako	10112557	20	Dako Envision Flex	Y	N	DAB
141	HIER	20	FE11	1:150	Dako	1016449	20	Polymer	Y	N	DAB
144	CC1	40 min	G219-1129	Pre-Dilute	Cell Marque	1529502G	16 min.	OptiView	No	Copper	DAB
145	CC1	40	G219-1129	1/400	CELLMARQUE	1313003A	24	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	FE11	RTU	Dako	10115752	20	EnVision Flex	Yes	No	DAB
175	HIER	32	G219-1129	Pre-dilute	Roche	1529502F	16	polymer	N	Y	DAB
181	HIER	20	FE11	1:150	DAKO	10106449	20	HRP-polymer	N	Y	DAB
186	HIER	20	G219-1129	1:200	Cell Marque	1529504A	15	LEICA BOND	N	N	DAB
189	CC1	40	G219-1129	Pre-dilute	Ventana	unknown	12	OptiView DAB	N	N	OptiView DAB
202	HIER citrate pH 9.5	20	25D12	1/50	Leica	6049441	15	Leica Refine detection kit	no	no	DAB
207	on line CC1	16 minutes	G219-1129	Prediluted	Cell Marque	1529502G	20	Ultraview	Y	Y	DAB
220	CC1	32min	G219-1129	Pre-Dilute	Cell Marque	1529502D	32min	OptiView	N	N	DAB
222	CC1	64	G219-1129	RTU	Ventana	1529502G	24	Optiview DAB	N	Y	Copper
231	CC1	64 mins	G219-1129	RTU	CELL MARQUE	1529502 C	20 mins	ULTRAVIEW (VENTANA)	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
101	CC1	32 min	EP49	1:100	Epitomics	EN072101	32min	Optiview	N	N	DAB
102	DAKO PT - HIGH PH	20	EP49	1:100	DAKO	10116602	30" RT	DAKO ENVISION FLEX	YES	YES	DAB+
103	CC1	64 MINS	44	PRE	VENTANA	F01005	32 MINS	OPTIVIEW	N	COPPER	DAB
104	HIER	20 min	EP49	RTU	DAKO	10116831	30 min	polymere	n	n	DAB
106	microwave pressure cooker	30	SP93	1:20	Cell Marque	1625006b	60	MACH4	no	no	DAB
107	Ultra cc1	32	EP49	1:150	Dako	10102831	32	Optiview DAB	N	Y	DAB
109	HIER high pH	64 MIN	44	RTU	VENTANA	G04117	20 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High ph 9.0	20 min @97C	EP49	1:200	DAKO	10116602	30 min	Dako Envision Flex	N	N	DAB
111	HIER CC1	48 min	SP93	1/100	Cell Marque	1512802B	32 min	optiview	N	Y	DAB
112	BOND Epitope Retrieval 2	40 minutes	EP49	1:1500	30 minutes	EN072101	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	64	EP49	1/200	Epitomics	C1090102	32	Optiview	N	Copper	DAB
115	Enviosion Flex High pH	30 mins	EP49	RTU	Dako	10114392	20 mins	Envision Flex	N	N	DAB
116	CC1	32 MIN	BC/44	1/400	BIOCARE MEDICALE	70814	48 MIN	OPTIVIEW DAB	N	.	DAB
124	CC1	32	EP49	1/50	Dako	10116602	32	DAB	n	n	DAB
126	Microwave pressure cooker with Citrate buffer pH 6.1	36	EP49	1:75	Dako	10116602	30	Quanto polymer	no	no	DAB+
138	High pH HIET	20	EP49	RTU	Dako	10116831	20	Dako Envision Flex	N	N	DAB
141	HIER	20	EP49	1:200	Dako	10116602	30	Polymer	Y	N	DAB
144	CC1	32 min	EP49	1:100	Cedarlane	ENO20910	32 min	OptiView	No	Copper	DAB
145	CC1	32	44	1/600	CELLMARQUE	1313501A	28	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	EP49	RTU	Dako	10116831	20	EnVision Flex	No	No	DAB
175	HIER	64	44	1 in 100	Cell Marque	1112208C	24	polymer	Y	Y	DAB
181	HIER	20	EP49	1:200	DAKO	10116602	30	HRP-polymer	N	N	DAB
186	HIER	20	BC/44	1:50	Biocare Medical	110315	15	LEICA BOND	Y	Y	DAB
189	CC1	64	SP93	Pre-dilute	Ventana	unknown	12	OptiView DAB	N	N	OptiView DAB
202	HIER citrate pH 9.5	40	PU29	1/25	ABCAM	GR262215	15	Leica Refine detection kit	no	no	DAB
207	CC1 on line	64 minutes	44	prediluted	Ventana	G04117	16 miutes	Ultraview DAB	N	Y	DAB
220	CC1	32min	EP49	1/50	DAKO	10114097	28min	OptiView	N	N	DAB
222	CC1	56	44	RTU	Ventana	G04117	16	Optiview DAB	Y	Y	Copper
231	CC1	64 mins	EP49	1/25	DAKO	10116602	32 mins	ULTRAVIEW (VENTANA)	Y	Y	DAB

Table S5. Descriptive statistics for MLH1 based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	25	84	21	20/21 (95%)	1	0.92	0.9
102	25	80	20	20/20 (100%)	1	1	1
103	25	76	19	19/19 (100%)	1	1	1
104	25	88	22	22/22 (100%)	1	1	1
106	25	92	23	23/23 (100%)	1	1	1
107	25	92	23	23/23 (100%)	1	1	1
109	25	84	21	21/21 (100%)	1	1	1
110	25	100	25	25/25 (100%)	1	1	1
111	25	96	24	24/24 (100%)	1	1	1
112	25	96	24	24/24 (100%)	1	1	1
114	25	88	22	22/22 (100%)	1	1	1
115	25	84	21	21/21 (100%)	1	1	1
116	25	84	21	21/21 (100%)	1	1	1
123	25	88	22	22/22 (100%)	1	1	1
124	25	60	15	15/15 (100%)	1	1	1
125	25	96	24	24/24 (100%)	1	1	1
126	25	88	22	22/22 (100%)	1	1	1
138	25	88	22	22/22 (100%)	1	1	1
141	25	92	23	23/23 (100%)	1	1	1
144	25	92	23	23/23 (100%)	1	1	1
145	25	96	24	24/24 (100%)	1	1	1
149	25	96	24	24/24 (100%)	1	1	1
175	25	92	23	23/23 (100%)	1	1	1
181	25	96	24	24/24 (100%)	1	1	1
186	25	92	23	23/23 (100%)	1	1	1
189	25	92	23	23/23 (100%)	1	1	1
202	25	84	21	21/21 (100%)	1	1	1
207	25	100	25	25/25 (100%)	1	1	1
217	25	96	24	24/24 (100%)	1	1	1
220	25	96	24	24/24 (100%)	1	1	1
222	25	92	23	23/23 (100%)	1	1	1
231	25	92	23	23/23 (100%)	1	1	1

Table S6. Descriptive statistics for PMS2 based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	25	84	21	21/21 (100%)	1	1	1
102	25	76	19	19/19 (100%)	1	1	1
103	25	80	20	20/20 (100%)	1	1	1
104	25	92	23	23/23 (100%)	1	1	1
106	25	92	23	23/23 (100%)	1	1	1
107	25	88	22	22/22 (100%)	1	1	1
109	25	84	21	21/21 (100%)	1	1	1
110	25	96	24	24/24 (100%)	1	1	1
111	25	96	24	24/24 (100%)	1	1	1
112	25	96	24	24/24 (100%)	1	1	1
114	25	96	24	24/24 (100%)	1	1	1
115	25	88	22	22/22 (100%)	1	1	1
116	25	84	21	21/21 (100%)	1	1	1
123	25	80	20	20/20 (100%)	1	1	1
124	25	80	20	20/20 (100%)	1	1	1
125	25	96	24	24/24 (100%)	1	1	1
126	25	92	23	23/23 (100%)	1	1	1
138	25	88	22	22/22 (100%)	1	1	1
141	25	88	22	22/22 (100%)	1	1	1
144	25	96	24	24/24 (100%)	1	1	1
145	25	92	23	23/23 (100%)	1	1	1
149	25	96	24	24/24 (100%)	1	1	1
175	25	88	22	22/22 (100%)	1	1	1
181	25	96	24	24/24 (100%)	1	1	1
186	25	96	24	24/24 (100%)	1	1	1
202	25	92	23	23/23 (100%)	1	1	1
207	25	96	24	22/24 (92%)	1	0.88	0.81
217	25	100	25	25/25 (100%)	1	1	1
220	25	96	24	24/24 (100%)	1	1	1
222	25	96	24	24/24 (100%)	1	1	1
231	25	76	19	19/19 (100%)	1	1	1

Table S7. Descriptive statistics for MSH2 based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	25	84	21	21/21 (100%)	1	1	1
102	25	88	22	22/22 (100%)	1	1	1
103	25	92	23	23/23 (100%)	1	1	1
104	25	92	23	23/23 (100%)	1	1	1
106	25	96	24	24/24 (100%)	1	1	1
107	25	84	21	21/21 (100%)	1	1	1
109	25	84	21	21/21 (100%)	1	1	1
110	25	100	25	25/25 (100%)	1	1	1
111	25	92	23	23/23 (100%)	1	1	1
112	25	96	24	24/24 (100%)	1	1	1
114	25	92	23	23/23 (100%)	1	1	1
115	25	92	23	23/23 (100%)	1	1	1
116	25	88	22	22/22 (100%)	1	1	1
123	25	84	21	21/21 (100%)	1	1	1
124	25	88	22	22/22 (100%)	1	1	1
125	25	92	23	23/23 (100%)	1	1	1
126	25	96	24	24/24 (100%)	1	1	1
138	25	100	25	25/25 (100%)	1	1	1
141	25	92	23	23/23 (100%)	1	1	1
144	25	100	25	25/25 (100%)	1	1	1
145	25	92	23	23/23 (100%)	1	1	1
149	25	96	24	24/24 (100%)	1	1	1
175	25	92	23	23/23 (100%)	1	1	1
181	25	96	24	24/24 (100%)	1	1	1
186	25	92	23	23/23 (100%)	1	1	1
189	25	96	24	24/24 (100%)	1	1	1
202	25	76	19	17/19 (89%)	0.94	0.5	0.44
207	25	96	24	23/24 (96%)	0.95	1	0.86
217	25	96	24	24/24 (100%)	1	1	1
220	25	92	23	23/23 (100%)	1	1	1
222	25	96	24	24/24 (100%)	1	1	1
231	25	92	23	23/23 (100%)	1	1	1

Table S8. Descriptive statistics for MSH6 based on cIQc assessment (cores 10 and 27 excluded due to observed patchy loss of MSH6 as a result of MLH1 loss described in the overview on page 1).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	23	82.61	19	18/19 (95%)	0.93	1	0.85
102	23	82.61	19	17/19 (89%)	0.93	0.75	0.68
103	23	82.61	19	17/19 (89%)	0.93	0.75	0.68
104	23	91.3	21	20/21 (95%)	0.94	1	0.88
106	23	91.3	21	20/21 (95%)	0.94	1	0.88
107	23	86.96	20	19/20 (95%)	0.93	1	0.87
109	23	78.26	18	17/18 (94%)	0.93	1	0.85
110	23	100	23	22/23 (96%)	0.94	1	0.89
111	23	95.65	22	20/22 (91%)	0.94	0.83	0.77
112	23	86.96	20	19/20 (95%)	0.94	1	0.86
114	23	95.65	22	22/22 (100%)	1	1	1
115	23	100	23	22/23 (96%)	0.94	1	0.89
116	23	86.96	20	19/20 (95%)	0.93	1	0.89
123	23	91.3	21	20/21 (95%)	0.94	1	0.88
124	23	86.96	20	19/20 (95%)	0.93	1	0.87
125	23	95.65	22	21/22 (95%)	0.94	1	0.89
126	23	95.65	22	21/22 (95%)	0.94	1	0.89
138	23	86.96	20	19/20 (95%)	0.93	1	0.87
141	23	91.3	21	20/21 (95%)	0.93	1	0.89
144	23	86.96	20	19/20 (95%)	0.93	1	0.87
145	23	91.3	21	20/21 (95%)	0.93	1	0.89
149	23	95.65	22	21/22 (95%)	0.94	1	0.89
175	23	91.3	21	20/21 (95%)	0.93	1	0.89
181	23	82.61	19	18/19 (95%)	0.93	1	0.85
186	23	95.65	22	21/22 (95%)	0.94	1	0.89
189	23	91.3	21	20/21 (95%)	0.94	1	0.88
202	23	86.96	20	19/20 (95%)	1	0.83	0.87
207	23	86.96	20	19/20 (95%)	0.94	1	0.86
217	23	95.65	22	21/22 (95%)	0.94	1	0.89
220	23	91.3	21	20/21 (95%)	0.94	1	0.88
222	23	95.65	22	21/22 (95%)	0.94	1	0.88
231	23	91.3	21	20/21 (95%)	0.94	1	0.88