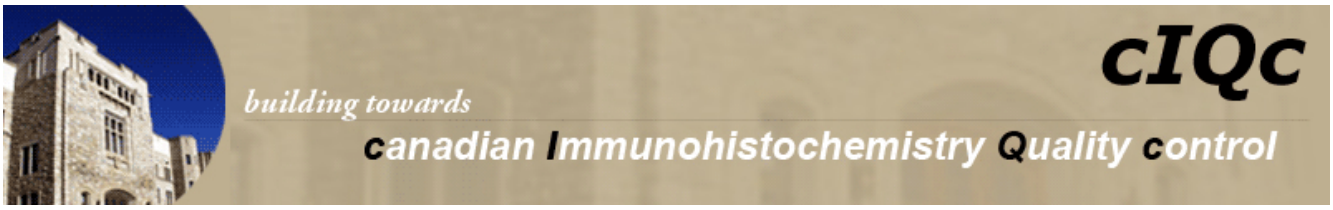

Assessors' report for cIQc Run 92: MMR Immunohistochemistry

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

Assessment performed on January 9 and February 8, 2019 at Vancouver General Hospital, Vancouver, BC

Overview

Run 92 MMR (MLH1, PMS2, MSH2 and MSH6) consisted of a 30 single-core tissue microarray of carcinomas with germline sequencing data to confirm MMR status. The MMR immunostains are always performed as part of a panel, and the unusual biology of MMR protein expression can cause problems for interpretation. For instance, although most tumours with absent MSH2 expression show absence of MSH6 expression, weak MSH6 expression may also be seen in association with MSH2 loss of expression and MSH2 germline mutations (*Pearlman R, Markow M, Knight D, Chen W, Arnold CA, Pritchard CC, Hampel H, Frankel WL. Two-stain immunohistochemical screening for Lynch syndrome in colorectal cancer may fail to detect mismatch repair deficiency. Mod Pathol. 2018 Dec 1;31:1891-900*). MMR immunohistochemistry works best on well-fixed small biopsy specimens (e.g. colonic or endometrial biopsies). Hysterectomy/colonic resection specimens, such as those used to create this tissue microarray, are usable but show heterogeneity of staining because of variable fixation.

For 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. 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 tissue core.

MLH1: MLH1 staining results were excellent. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Adequate	Weak granular nuclear staining in Core 22
106	Optimal	
107	Optimal	
109	Optimal	
110	Optimal	Nice staining
111	Optimal	Intense staining compared to other labs
112	Optimal	
113	Optimal	Very weak granular staining in Core 22
114	Optimal	
125	Optimal	
136	Optimal	
138	Optimal	
141	Optimal	Nice staining
144	Optimal	
149	Optimal	

Lab ID	IHC Status*	Comment
175	Optimal	
181	Optimal	
186	Optimal	Cytoplasmic background staining in stromal cells but still easily interpretable
189	Optimal	
190	Optimal	
193	Optimal	
194	Optimal	
202	Optimal	Cytoplasmic blush
207	Optimal	
217	Optimal	
220	Optimal	Cytoplasmic blush
222	Optimal	
230	Optimal	
231	Optimal	Cytoplasmic blush
236	Optimal	

*Based on cIQc assessor consensus

Lab/ Core	101	102	106	107	109	110	111	112	113	114	125	136	138	141	144	149	175	181	186	189	190	193	194	202	207	217	220	222	230	231	236	MMR Status
1	E	E	E	E	E	E	E	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
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	MLH1
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	E	E	E	E	E	E	E	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	E	E	E	E	E	E	E	Normal
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	Normal
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	MSH6
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	E	E	E	E	E	E	Normal
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	MSH6
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	E	E	E	E	E	E	E	PMS2
10	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	U	E	E	U	U	E	E	E	U	U	U	E	E	E	U	Normal
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	Normal
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	A	A	A	A	A	A	A	MLH1
13	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	U	E	E	E	E	U	MSH2
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	E	E	E	E	E	E	E	MSH2
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	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	A	A	A	A	A	A	A	MLH1
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
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	E	E	E	E	E	E	E	MSH2
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
20	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	E	E	E	E	Normal
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	MSH6
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	A	A	A	A	A	MLH1
23	E	E	U	U	U	E	E	E	E	E	U	E	E	U	U	U	U	U	U	U	U	U	E	U	U	U	E	E	E	E	U	MSH2
24	E	E	E	E	E	E	E	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
25	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
26	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	MSH2
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	E	E	E	E	E	E	E	MSH6
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
29	E	E	E	E	E	E	E	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
30	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

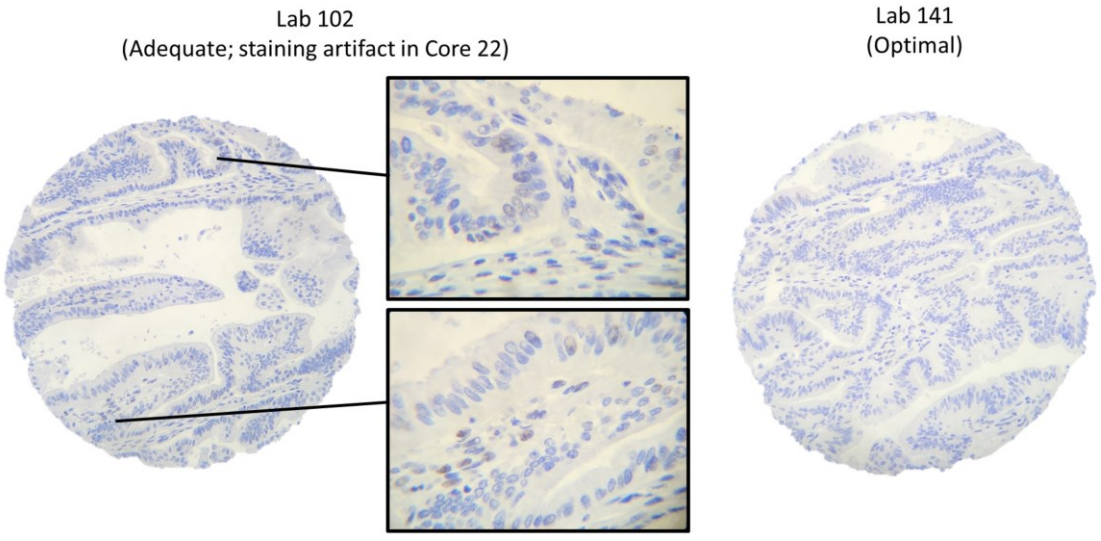

Figure 1. Representative images of an MLH1-deficient case (Core 22) that showed weak granular nuclear staining in Lab 102 but not by other participants.

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

Lab ID	IHC Status*	Comment
101	Optimal	
102	Adequate	Very weak granular nuclear staining
106	Optimal	
107	Optimal	Slightly weak tumour cell staining
109	Optimal	
109n	Optimal	New protocol has minute differences from old protocol
110	Optimal	
111	Optimal	Cytoplasmic blush
112	Optimal	
113	Optimal	
114	Optimal	
125	Optimal	Cytoplasmic blush
136	Optimal	
138	Optimal	
141	Optimal	
144	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Optimal	
189	Adequate	Generally weak staining
190	Adequate	Granular cytoplasmic background
193	Optimal	
194	Optimal	
202	Optimal	Strong cytoplasmic blush
207	Optimal	
217	--	Slide not available for assessment
220	Optimal	
222	Sub-optimal	Very intense staining that makes interpretation challenging
230	Optimal	
231	Optimal	Cytoplasmic blush
236	Adequate	Strong granular cytoplasmic blush that generally makes interpretation more challenging

*Based on cIQc assessor consensus

Lab/ Core	101	102	106	107	109	110	111	112	113	114	125	136	138	141	144	149	175	181	186	189	190	193	194	202	207	217	220	222	230	231	236	MMR Status
1	E	E	E	E	E	E	E	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
2	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	A	MLH1
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	E	E	E	E	E	E	E	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	E	E	E	E	E	E	E	Normal
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	Normal
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	MSH6
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	E	E	E	E	E	E	Normal
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	MSH6
9	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	A	A	A	PMS2
10	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	U	E	E	E	E	E	E	Normal
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	Normal
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	A	A	A	A	A	A	A	MLH1
13	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	E	E	E	MSH2
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	E	E	E	E	E	E	E	MSH2
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	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	A	A	A	A	A	A	A	MLH1
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
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	E	E	E	E	E	E	E	MSH2
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
20	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	E	E	E	E	Normal
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	A	E	E	E	MSH6
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	A	A	A	A	A	MLH1
23	U	U	U	U	U	E	E	E	U	U	E	E	E	E	E	U	U	U	U	E	U	U	U	U	U	U	U	U	E	U	E	MSH2
24	E	E	E	E	E	E	E	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
25	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	E	Normal
26	E	E	E	E	E	E	E	U	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	MSH2
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	E	E	E	E	E	E	E	MSH6
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
29	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
30	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

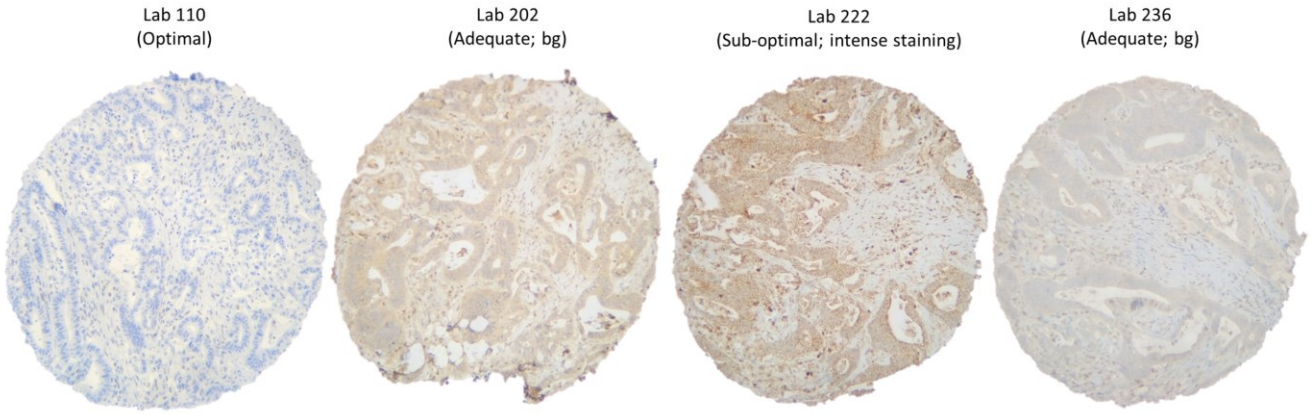

Figure 2. Representative staining of Core 29, a PMS2-deficient case, by different participants. (bg = background)

MSH2: Overall MSH2 staining results were excellent. Core 21 was a failed result for several labs primarily due to the tissue core lacking benign components as internal controls (i.e. all tumour cells). Core 30 had generally weak staining in tumour cells due to poor fixation, leading to variable results. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	
125	Optimal	
136	Optimal	
138	Optimal	
141	Adequate	Genearlly weak staining
144	Adequate	High background
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	Weak staining
189	Optimal	
190	Optimal	
193	Adequate	Weak staining
194	Optimal	
202	Adequate	Protocol validation in progress
207	Optimal	
217	Optimal	Slight background
220	Optimal	
222	Optimal	
230	Optimal	Nice staining
231	Optimal	
236	Optimal	

*Based on cIQc assessor consensus

Lab/ Core	101	102	106	107	109	110	111	112	113	114	125	136	138	141	144	149	175	181	186	189	190	193	194	202	207	217	220	222	230	231	236	MMR Status
1	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
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	MLH1
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	A	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	E	E	E	E	E	E	E	E	Normal
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	Normal
6	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	E	MSH6
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	E	E	E	E	E	E	Normal
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	MSH6
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	E	E	E	E	E	E	E	PMS2
10	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	U	E	U	E	E	E	E	Normal
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	Normal
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	MLH1
13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	U	U	A	A	U	A	A	A	A	A	A	A	MSH2
14	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
15	E	E	E	E	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	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	E	E	E	E	E	E	E	MLH1
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
18	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
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
20	U	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	E	E	E	E	Normal
21	F	E	E	U	F	E	E	E	E	E	E	E	E	F	E	E	E	E	F	F	F	F	F	E	E	E	E	E	E	E	E	MSH6
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	MLH1
23	U	A	U	A	U	A	U	A	U	A	U	A	U	A	U	U	U	U	U	U	U	U	A	U	U	U	U	A	A	U	A	MSH2
24	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
25	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
26	A	A	A	A	A	A	A	U	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	MSH2
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	E	E	E	E	E	E	E	MSH6
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
29	E	E	E	E	E	E	E	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
30	E	E	E	A	A	E	E	E	E	E	E	E	E	A	F	E	E	E	F	E	E	F	E	E	E	E	E	E	E	E	E	MLH1

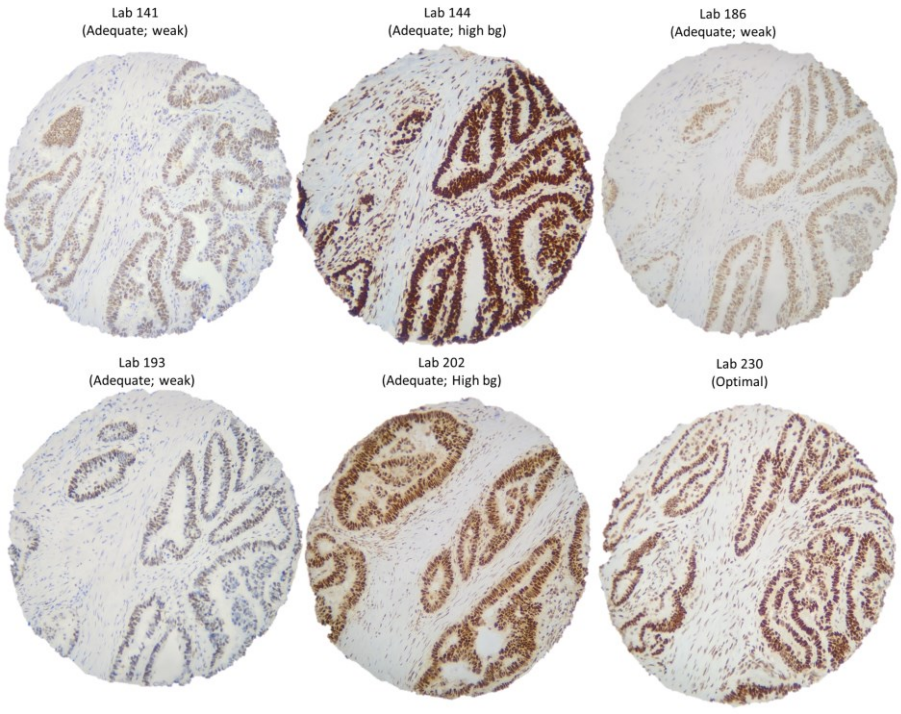

Figure 3. Representative staining of Core 7, a case expressing MSH2, by different participants. (bg = background)

MSH6: MSH6 staining results were very good, overall. Cores 1, 18 and 24 in particular showed variable and weak MSH6 staining that can be seen with MSH2 loss**.** Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
109n	Optimal	New protocol has greater intensity and slight granular background staining compared to old protocol
110	Optimal	
111	Optimal	
112	Sub-optimal	Very weak staining
113	Optimal	
114	Optimal	
125	Optimal	
136	Optimal	
138	Optimal	
141	Optimal	Nice staining
144	Optimal	Intense staining
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	Weak staining
189	Optimal	
190	Adequate	Generally weak staining
193	Optimal	
194	Optimal	
202	Failed	Protocol validation in progress
207	Optimal	
217	Optimal	
220	Optimal	
222	Optimal	
230	Optimal	
231	Optimal	
236	Optimal	

*Based on cIQc assessor consensus

Lab/ Core	101	102	106	107	109	110	111	112	113	114	125	136	138	141	144	149	175	181	186	189	190	193	194	202	207	217	220	222	230	231	236	MMR Status
1	E	E	E	A	A	E	E	A	E	E	E	A	E	E	E	E	A	A	A	E	A	E	A	E	A	E	E	A	E	F	E	MSH2
2	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	MLH1
3	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	A	E	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	E	E	E	E	E	E	E	E	Normal
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	Normal
6	A	A	A	F	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	U	F	A	A	A	A	A	U	A	A	U	A	MSH6
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	E	E	E	E	E	E	Normal
8	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	MSH6
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	E	E	E	E	E	E	E	PMS2
10	E	E	U	E	E	E	E	U	E	E	E	E	E	E	E	E	E	U	E	E	U	E	U	E	U	E	U	E	E	U	E	Normal
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	Normal
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	MLH1
13	A	A	A	A	A	A	A	U	A	A	A	A	E	A	A	U	A	U	A	A	U	A	A	E	A	U	A	A	A	A	U	MSH2
14	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	A	MSH2
15	A	A	A	U	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	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	E	E	E	E	E	E	E	MLH1
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
18	E	E	A	A	A	A	E	A	A	A	A	A	A	A	E	A	A	A	A	A	A	A	A	E	A	E	A	A	A	A	A	MSH2
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
20	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	Normal
21	A	F	F	U	A	A	A	F	A	A	A	A	A	A	A	F	A	F	F	A	F	F	F	A	F	A	F	A	E	F	A	MSH6
22	E	E	E	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	MLH1
23	A	U	U	A	U	A	U	U	U	U	U	A	A	A	U	U	U	U	U	U	U	A	U	U	U	U	U	U	A	U	U	MSH2
24	A	E	A	A	A	A	A	F	A	A	E	A	A	A	A	E	A	A	A	E	A	A	A	E	A	E	A	A	A	A	E	MSH2
25	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
26	A	A	A	A	A	A	A	F	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	U	A	A	MSH2
27	A	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	A	A	A	A	MSH6
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	Normal
29	E	E	E	E	E	E	E	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
30	E	E	E	F	E	E	E	F	E	E	E	A	E	E	E	E	E	E	A	E	A	E	E	E	E	E	E	E	E	E	E	MLH1

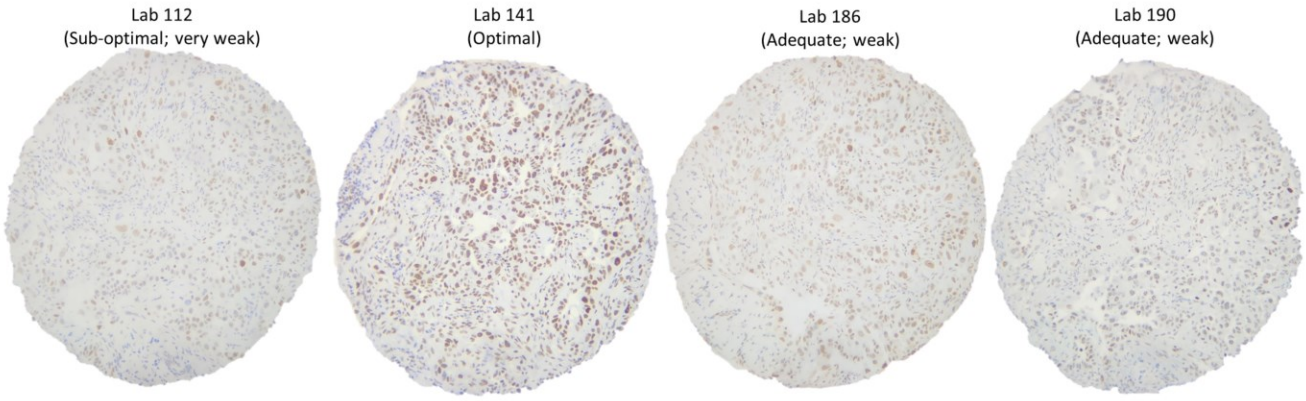

Figure 4. Representative staining of Core 17, a case expressing MSH6, by different participants.

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. Supplementary Table 9 provides the definitions of IHC Status and recommended participant action. 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	EnV FlexTRS, HIgh PH	30 min	ES05	RTU	Dako	10134314	20 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	ES05	1:80	DAKO	10134323	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
106	microwave/pressure	30	ES05	1:20	Novacastra	6056865	45	MACH4	no	no	DAB
107	ultra cc1	64	ES05	1:20	Leica/Novocastra	6044922	40	Optiview DAB	N	Y	DAB
109	HIER high pH CC1	32 MIN	M1	RTU	ROCHE	E05574	16 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	ES05	1:100	DAKO	10136692	20 min	Dako Envision Flex	Y	N	DAB
111	HIER	48	G168-15	1/50	BIOCARE	61317R	32	OPTIVIEW	Y	Y	DAB
112	Bond Epitope Retrieval 2 pH 9.0	25 minutes	ES05	1:100	Leica (Novocastra)	6052436	30 minutes	BOND polymer refine	none	none	DAB
113	Dako High pH	30min	ES05	Predilute	Dako	10131691	20min	Flex+30	Yes	No	DAB
114	Envision Flex TRS, High pH	30	ES05	RTU	DAKO	10125751	20	DAKO OMNIS Envision Flex	N	Mouse Linker	Envision Flex DAB
136	DAKO PT HIGH PH	20	ES05	RTU	DAKO	10131691	15	DAKO ENVISION FLEX +	Y	N	DAB
138	EDTA HIER	20	ES05	RTU	Dako	10138487	20	Polymer	Y	N	DAB
141	HIER	20	ES05	1:100	DAKO	10136692	20	POLYMER	Y	N	DAB
144	CC1	56 min.	ES05	1:25	Novocastra	6053681	32 min.	Opti-view	No	Copper	DAB
149	high pH OMNIS	30 min at 97 C	ES05	RTU	Dako Agilent	10138487	20	EnVision Flex OMNIS	Yes	No	DAB
175	HIER	64	M1	Pre dilute	Roche	E08159	16	Polymer (opti-dab)	n	y	DAB
181	HIER pH 9	20	ESO5	1:100	DAKO	10136692	20	HRP-POLYMER	Y	N	DAB
186	HIER	20	G168-728	1:50	CELL MARQUE	1630905A	15	POLYMER	N	N	DAB
189	CC1	64	M1	pre-dilute	Ventana	unknown	24	OptiView DAB	N	N	OptiView DAB
190	CC1	40	ES05	1:50	Dako	10127457	32	Ventana Optiview	Y	N	DAB
193	HIER Low pH Omnis	30 Min.	ES05	RTU	DAKO	10139638	30 Min	Envision Flex	Yes	No	DAB
194	CC1	64	M1	RTU	ROCHE/VENTANA	E08159	16	OPTIVIEW	N	N	DAB
202	ER2	20	ES05	1/10	BD Pharmagen	3256964	15	Refine Detection system	no	no	DAB
207	Env Flex TRS High PH	25 minutes	ES05	RTU	Agilent	IR079	25 minutes	Envision Flex DAB	Y	Y	DAB
217	HIER CC1	56	G219-1129	RTU	Roche Ventana	E12501	32	Optiview	N	Y	DAB
220	CC1	72min	ES05	1/25	DAKO	10128797	1hr	OptiView	N	N	DAB
222	Ultra CC1	40	M1	RTU	Ventana	E05574	4	Optiview DAB	Y	Y	Copper
230	HIER	64	ES05	predilute	DAKO	10140510	40	Optiview	Y	N	DAB
231	HIER	64 mins	M1	RTU	ROCHE/VENTANA	E12501	40 mins	ULTRAVIEW (VENTANA)	Y	Y	DAB
236	CC1	64	ES05	1:20	DAKO	10100344	40	OptiView DAB	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	EnV FlexTRS, HIgh PH	30 min	EP51	RTU	Dako	10133142	20 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	EP51	1:20	DAKO	10137457	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
106	microwave/pressure	30	MRQ-28	1:35	Cell Marque	19554	45	MACH4	no	yes	DAB
107	Decloak FLEX TRS High pH	120 celcius 30 sec	A16-4	1:200	BD Biosciences	8121982	30	FLEX 30+	N	N	DAB
109	HIER high pH CC1	40 MIN	EPR3947	RTU	CELL MARQUE	V0001198	48 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	EP51	1:50	DAKO	10132271	30 min	Dako Envision Flex	N	N	DAB
111	HIER	56	EP51	1/25	DAKO	10140542	32	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	30 minutes	EP51	1:75 using DAKO background reducing diluent	DAKO	10137457	30 minutes	BOND polymer refine	none	none	DAB
113	Dako High pH	20min	A16-4	Predilute	Dako	8121982	25min	Flex+20	Y	N	DAB
114	Envision Flex TRS, High pH	30	EP51	RTU	DAKO	10134727	20	DAKO OMNIS Envision Flex	N	Rabbit Linker	Envision Flex DAB
136	DAKO PT HIGH PH	20	EP51	RTU	DAKO	10131396	30	DAKO ENVISION FLEX +	N	N	DAB
138	EDTA HIER	20	EP51	RTU	Dako	10138754	30	Polymer	Y	N	DAB
141	HIER	20	EP51	1:50	DAKO	10132271	30	POLYMER	Y	N	DAB
144	CC1	64 min.	EPR3947	Pre-Dilute	Cell Marque	V1198	20 min.	Opti-view	Yes	Copper	DAB
149	high pH OMNIS	30 min at 97 C	EP51	RTU	Dako Agilent	10138754	20	EnVision Flex OMNIS	Yes	No	DAB
175	HIER	64	EPR3947	Pre dilute	roche	V0001218	32	Polymer (opti-DAB)	y	y	DAB
181	HIER pH 9	20	EP51	1:50	DAKO	10132271	30	HRP-POLYMER	N	N	DAB
186	HIER	20	ERP3947	1:4	CELL MARQUE	20343	15	POLYMER	N	N	DAB
189	CC1	92	A16-4	pre-dilute	Ventana	unknown	32	OptiView DAB	Y	N	OptiView DAB
190	CC1	32	EPR3947	Pre-dilute	Cell Marque	V0000884	32	Ventana Optiview	Y	N	DAB
193	HIER High pH Omnis	30 Min.	EP51	RTU	DAKO	10138754	30 Min	Envision Flex	Yes	No	DAB
194	CC1	64	EPR3497	RTU	CELL MARQUE / ROCHE	V0001198	32	OPTIVIEW	N	N	DAB
202	ER2	30min	a16-4	1/25	BD Pharmagen	331643	15	Refine Detection system	no	no	DAB
207	Env Flex TRS High PH	20 minutes	EP51	RTU	Agilent	10138754	20 minutes	Envision Flex	Y	Y	DAB
217	HIER CC1	40	EPR3947	RTU	Roche Ventana	V0001253	48	Optiview	Y	Y	DAB
220	CC1	64min	EP51	1/40	DAKO	10132224	1h8min	OptiView	N	N	DAB
222	Ultra CC1	92	A16-4	RTU	Ventana	Y24627Z	44	Optiview DAB	Y	Y	Copper
230	HIER	64	EPR3947	predilute	Ventana	V0001254	32	Optiview	Y	N	DAB
231	HIER	64 mins	EP51	1/25	DAKO	10140542	1 HR 20 MINS	ULTRAVIEW (VENTANA)	Y	Y	DAB
236	CC1	64	EPR3947	RTU	ROCHE	1506813C	48	OptiView DAB	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	EnV FlexTRS, HIgh PH	30 min	FE11	RTU	Dako	10124949	30 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	FE11	1:40	DAKO	10134927	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
106	microwave/pressure	30	FE11	1:150	Agillent	10140107	60	MACH4	no	yes	DAB
107	cc1	48	G219-1129	1:200	ESBE/Cell Marque	1620915E	32	Optiview DAB	N	Y	DAB
109	HIER high pH CC1	32 MIN	G219-1129	RTU	CELL MARQUE	V0001056	8 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	FE11	1:300	DAKO	10134927	20 min	Dako Envision Flex	Y	N	DAB
111	HIER	40	G219-1129	1/100	CELL MARQUE	1616010A	32	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	30 minutes	FE11	1:100	DAKO	10137646	30 minutes	BOND polymer refine	none	none	DAB
113	Dako High pH	20min	FE11	Predilute	Dako	10138657	20min	Flex+20	Y	N	DAB
114	Envision Flex TRS, High pH	30	FE11	RTU	DAKO	10133138	30	DAKO OMNIS Envision Flex	N	N	Envision Flex DAB
136	DAKO PT HIGH PH	20	FE11	RTU	DAKO	101478	30	DAKO ENVISION FLEX +	N	N	DAB
138	EDTA HIER	20	FE11	RTU	Dako	10138657	20	Polymer	N	N	DAB
141	HIER	20	FE11	1:150	DAKO	10134927	20	POLYMER	Y	N	DAB
144	CC1	64 min	G219-1129	Pre-Dilute	Cell Marque	V982	24 min.	Opti-view	Yes	Copper	DAB
149	high pH OMNIS	30 min at 97 C	FE11	RTU	Dako Agilent	10134390	30	EnVision Flex OMNIS	No	No	DAB
175	HIER	32	g219-1129	Pre dilute	Roche	V0001056	16	Polymer (opti-DAB)	n	y	DAB
181	HIER pH 9	20	FE11	1:300	DAKO	10134927	20	HRP-POLYMER	Y	N	DAB
186	HIER	20	G219-1129	1:200	CELL MARQUE	1620915E	15	POLYMER	N	N	DAB
189	CC1	40	G219-1129	pre-dilute	Ventana	unknown	12	OptiView DAB	N	N	OptiView DAB
190	CC1	32 (mild)	G219-1129	1:100	Cell Marque	5920	32	Ventana iView	N	N	DAB
193	HIER High pH Omnis	30 Min.	FE11	RTU	DAKO	10133138	30 Min	Envision Flex	No	No	DAB
194	CC1	32	G219-1129	RTU	CELL MARQUE / ROCHE	V0001243	16	OPTIVIEW	N	N	DAB
202	ER2	20	G219-1129	1/100	Cell marque	20822	15	Refine Detection system	no	no	DAB
207	Env Flex TRS High PH	30 minutes	FE11	RTU	Agilent	10138657	30 minutes	Envision Flex	N	Y	DAB
217	HIER CC1	56	G219-1129	RTU	Roche Ventana	V0001230	32	Optiview	N	Y	DAB
220	CC1	32min	G219-1129	Pre-Dilute	Cell Marque	V0000981	32min	OptiView	N	N	DAB
222	ultra CC1	64	G219-1129	RTU	Ventana	V0001055	24	Optiview DAB	N	Y	Copper
230	HIER	64	G219-1129	predilute	Ventana	V001243	32	Optiview	N	N	DAB
231	HIER	64 mins	G219-1129	RTU	CELL MARQUE	1616008 B	20 mins	ULTRAVIEW (VENTANA)	Y	Y	DAB
236	CC1	40	G219-1129	RTU	ROCHE	1529502C	20	OptiView DAB	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	EnV FlexTRS, HIgh PH	30 min	EP49	RTU	Dako	10134226	20 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	EP49	1:100	DAKO	10128801	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
106	microwave/pressure	20	SP93	1:80	Cell Marque	9803	45	MACH4r	no	yes	DAB
107	ultra cc1	56	EPR 3945	1:750	Abcam	GR262215-19	32	Optiview DAB	N	Y	DAB
109	HIER high pH CC1	56 MIN	44	RTU	ROCHE	E06199	16 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	EP49	1:200	DAKO	10134759	30 min	Dako Envision Flex	N	N	DAB
111	HIER	48	SP93	1/100	CELL MARQUE	9803	32	OPTIVIEW	N	Y	DAB
112	BOND Epitope Retrieval 2 pH 9.0	40 minutes	EP49	1:1500	Epitomics/cell Marque	EP062207	30 minutes	BOND polymer refine	none	none	DAB
113	Dako High pH	30min	EP49	Predilute	Dako	10140060	20min	Flex20	N	N	DAB
114	Envision Flex TRS, High pH	30	EP49	RTU	DAKO	10134226	20	DAKO OMNIS Envision Flex	N	N	Envision Flex DAB
136	DAKO PT HIGH PH	20	EP49	RTU	DAKO	10132018	10	DAKO ENVISION FLEX +	N	N	DAB
138	EDTA HIER	20	EP49	RTU	Dako	10138279	20	Polymer	Y	N	DAB
141	HIER	20	FE49	1:200	DAKO	10134759	30	POLYMER	Y	N	DAB
144	CC1	40 min.	EP49	1:25	Dako	10137455	24 min.	Opti-view	Yes	Copper	DAB
149	high pH OMNIS	30 min at 97 C	EP49	RTU	Dako Agilent	10134226	20	EnVision Flex OMNIS	No	No	DAB
175	HIER	64	44	1 in 100	Cell Marque	1510722B	24	Polymer (opti-DAB)	y	y	DAB
181	HIER pH 9	20	EP49	1:200	DAKO	10134759	30	HRP-POLYMER	N	N	DAB
186	HIER	20	44	1:100	BIOCARE MEDICAL	31017	15	POLYMER	N	N	DAB
189	CC1	64	SP93	pre-dilute	Ventana	unknown	12	OptiView DAB	N	N	OptiView DAB
190	CC1	32 (mild)	EP49	1:100	Epitomics	EN020910	32	Ventana Optiview	N	N	DAB
193	HIER High pH Omnis	30 Min.	EP49	RTU	DAKO	10135789	20 Min.	Envision Flex	No	No	DAB
194	CC1	63	44	RTU	ROCHE/VENTANA	E12802	16	OPTIVIEW	N	N	DAB
202	ER2	40	AB92471	1/25	ABCAM	20822	15	Refine Detection system	no	no	DAB
207	Env Flex TRS High PH	20 miutes	Ep49	RTU	Agilent	10130279	20 minutes	Dako Envision Flex	N	Y	DAB
217	HIER CC1	64	GTBP45	1:3000	Roche Ventana	130807D	60	Optiview	N	Y	DAB
220	CC1	32min	EP49	1/50	DAKO	10116602	28min	OptiView	N	N	DAB
222	Ultra CC1	64	SP93	RTU	Ventana	Y18767Z	12	Optiview DAB	N	Y	Copper
230	HIER	64	EP49	predilute	DAKO	10142494	32	Optiview	N	N	DAB
231	HIER	64 mins	EP49	1/25	DAKO	10140335	32 mins	ULTRAVIEW (VENTANA)	Y	Y	DAB
236	CC1	40	EP49	1:20	DAKO	10100348	40	OptiView DAB	N	N	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	30	100	30	30/30 (100%)	1	1	1
102	30	100	30	29/30 (97%)	1	0.8	0.87
106	30	96.67	29	29/29 (100%)	1	1	1
107	30	96.67	29	29/29 (100%)	1	1	1
109	30	96.67	29	29/29 (100%)	1	1	1
110	30	100	30	30/30 (100%)	1	1	1
111	30	100	30	30/30 (100%)	1	1	1
112	30	93.33	28	28/28 (100%)	1	1	1
113	30	100	30	30/30 (100%)	1	1	1
114	30	100	30	30/30 (100%)	1	1	1
125	30	96.67	29	29/29 (100%)	1	1	1
136	30	96.67	29	29/29 (100%)	1	1	1
138	30	100	30	30/30 (100%)	1	1	1
141	30	96.67	29	29/29 (100%)	1	1	1
144	30	96.67	29	29/29 (100%)	1	1	1
149	30	96.67	29	29/29 (100%)	1	1	1
175	30	93.33	28	28/28 (100%)	1	1	1
181	30	93.33	28	28/28 (100%)	1	1	1
186	30	96.67	29	29/29 (100%)	1	1	1
189	30	93.33	28	28/28 (100%)	1	1	1
190	30	90	27	27/27 (100%)	1	1	1
193	30	96.67	29	29/29 (100%)	1	1	1
194	30	100	30	30/30 (100%)	1	1	1
202	30	96.67	29	29/29 (100%)	1	1	1
207	30	93.33	28	28/28 (100%)	1	1	1
217	30	90	27	27/27 (100%)	1	1	1
220	30	96.67	29	29/29 (100%)	1	1	1
222	30	100	30	30/30 (100%)	1	1	1
230	30	100	30	30/30 (100%)	1	1	1
231	30	100	30	30/30 (100%)	1	1	1
236	30	90	27	27/27 (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	30	93.33	28	28/28 (100%)	1	1	1
102	30	96.67	29	28/29 (97%)	1	0.86	0.9
106	30	96.67	29	29/29 (100%)	1	1	1
107	30	96.67	29	29/29 (100%)	1	1	1
109	30	96.67	29	29/29 (100%)	1	1	1
110	30	100	30	30/30 (100%)	1	1	1
111	30	100	30	30/30 (100%)	1	1	1
112	30	90	27	27/27 (100%)	1	1	1
113	30	96.67	29	29/29 (100%)	1	1	1
114	30	96.67	29	29/29 (100%)	1	1	1
125	30	100	30	30/30 (100%)	1	1	1
136	30	96.67	29	29/29 (100%)	1	1	1
138	30	100	30	30/30 (100%)	1	1	1
141	30	100	30	30/30 (100%)	1	1	1
144	30	100	30	30/30 (100%)	1	1	1
149	30	96.67	29	29/29 (100%)	1	1	1
175	30	96.67	29	29/29 (100%)	1	1	1
181	30	96.67	29	29/29 (100%)	1	1	1
186	30	96.67	29	29/29 (100%)	1	1	1
189	30	100	30	30/30 (100%)	1	1	1
190	30	90	27	27/27 (100%)	1	1	1
193	30	96.67	29	29/29 (100%)	1	1	1
194	30	93.33	28	28/28 (100%)	1	1	1
202	30	93.33	28	28/28 (100%)	1	1	1
207	30	93.33	28	28/28 (100%)	1	1	1
217	30	96.67	29	29/29 (100%)	1	1	1
220	30	96.67	29	29/29 (100%)	1	1	1
222	30	93.33	28	27/28 (96%)	0.95	1	0.9
230	30	96.67	29	29/29 (100%)	1	1	1
231	30	96.67	29	29/29 (100%)	1	1	1
236	30	100	30	30/30 (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	30	90	27	27/27 (100%)	1	1	1
102	30	100	30	30/30 (100%)	1	1	1
106	30	96.67	29	29/29 (100%)	1	1	1
107	30	96.67	29	28/29 (97%)	0.95	1	0.92
109	30	93.33	28	27/28 (96%)	0.95	1	0.91
110	30	100	30	30/30 (100%)	1	1	1
111	30	96.67	29	29/29 (100%)	1	1	1
112	30	90	27	27/27 (100%)	1	1	1
113	30	96.67	29	29/29 (100%)	1	1	1
114	30	100	30	30/30 (100%)	1	1	1
125	30	96.67	29	29/29 (100%)	1	1	1
136	30	96.67	29	29/29 (100%)	1	1	1
138	30	96.67	29	29/29 (100%)	1	1	1
141	30	96.67	29	28/29 (97%)	0.95	1	0.92
144	30	90	27	27/27 (100%)	1	1	1
149	30	96.67	29	29/29 (100%)	1	1	1
175	30	96.67	29	29/29 (100%)	1	1	1
181	30	96.67	29	29/29 (100%)	1	1	1
186	30	90	27	26/27 (96%)	0.95	1	0.91
189	30	90	27	27/27 (100%)	1	1	1
190	30	86.67	26	26/26 (100%)	1	1	1
193	30	90	27	27/27 (100%)	1	1	1
194	30	96.67	29	29/29 (100%)	1	1	1
202	30	90	27	27/27 (100%)	1	1	1
207	30	93.33	28	28/28 (100%)	1	1	1
217	30	96.67	29	29/29 (100%)	1	1	1
220	30	93.33	28	28/28 (100%)	1	1	1
222	30	100	30	30/30 (100%)	1	1	1
230	30	96.67	29	29/29 (100%)	1	1	1
231	30	96.67	29	29/29 (100%)	1	1	1
236	30	100	30	30/30 (100%)	1	1	1

Table S8. Descriptive statistics for MSH6 based on cIQc assessment (Cores 1, 18 and 24 were excluded due to variable weak expression associated with loss of MSH2).

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	27	96.3	26	26/26 (100%)	1	1	1
102	27	88.89	24	23/24 (96%)	1	0.88	0.9
106	27	88.89	24	24/24 (100%)	1	1	1
107	27	85.19	23	23/23 (100%)	1	1	1
109	27	96.3	26	26/26 (100%)	1	1	1
110	27	100	27	27/27 (100%)	1	1	1
111	27	96.3	26	26/26 (100%)	1	1	1
112	27	62.96	17	17/17 (100%)	1	1	1
113	27	96.3	26	26/26 (100%)	1	1	1
114	27	96.3	26	26/26 (100%)	1	1	1
125	27	96.3	26	26/26 (100%)	1	1	1
136	27	96.3	26	25/26 (96%)	0.94	1	0.92
138	27	100	27	26/27 (96%)	1	0.9	0.92
141	27	100	27	27/27 (100%)	1	1	1
144	27	96.3	26	26/26 (100%)	1	1	1
149	27	88.89	24	24/24 (100%)	1	1	1
175	27	96.3	26	26/26 (100%)	1	1	1
181	27	85.19	23	23/23 (100%)	1	1	1
186	27	88.89	24	23/24 (96%)	0.94	1	0.91
189	27	92.59	25	25/25 (100%)	1	1	1
190	27	77.78	21	20/21 (95%)	0.94	1	0.88
193	27	96.3	26	26/26 (100%)	1	1	1
194	27	88.89	24	24/24 (100%)	1	1	1
202	27	96.3	26	22/26 (85%)	1	0.56	0.62
207	27	88.89	24	24/24 (100%)	1	1	1
217	27	92.59	25	24/25 (96%)	1	0.88	0.9
220	27	85.19	23	23/23 (100%)	1	1	1
222	27	96.3	26	26/26 (100%)	1	1	1
230	27	96.3	26	25/26 (96%)	1	0.89	0.91
231	27	85.19	23	23/23 (100%)	1	1	1
236	27	92.59	25	25/25 (100%)	1	1	1

Table S8. cIQc Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	cIQc Proficiency Testing Performance
Optimal	The staining was considered of the highest technical quality to allow for accurate readout of the target biomarker.	PASS
Adequate	The staining was considered to be sufficient for the purpose of accurate readout of the target biomarker.	PASS
Sub-optimal	The staining was considered to be of a quality that makes readout of the test challenging, which may lead to inaccurate readout of the target biomarker.	PASS, CONDITIONALLY[1]
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	FAIL[2]

1 – A one-time suboptimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".
1,2 – Please contact the cIQc for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.