Assessor's report for cIQc Run 31: MMR enzymes (MLH1, MSH2, MSH6 and PMS2)

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Assessment performed on Tuesday, October 22, 2013, at Vancouver General Hospital

Overview

The 2013 cIQc MMR enzyme challenge asked participating laboratories to stain a 31-case colorectal carcinoma tissue microarray for MLH1, MSH2, MSH6 and PMS2. Overall, self-assessments from participating labs were very good. All participating laboratories except Labs 123 and 200 returned slides to cIQc in time for the assessment meeting in Vancouver, and all slides were blindly reviewed by four assessors (BG, KO, ZK and JG). Independent review led to infrequent alteration of original self-reported results for certain discordant cores due to 1) an obvious data entry error or 2) a core was unanimously agreed to have a different interpretation after final cIQc review than self-assessment.

MLH1: Cores 6, 8, 11, 18, 22, 24 and 29 were excluded from all statistical analyses for three possible reasons: 1) a core was determined to have a highly heterogeneous tumor content across participating labs upon independent review, 2) more than 50% of participating laboratories experienced core dropout/staining failure for a single core and reported an unsatisfactory/failed score or 3) extensive necrosis was observed for most participating labs (occasionally leading to an unusual coarse granular staining pattern).

After cIQc independent review, the majority of participating laboratories were observed to have adequate MLH1 staining. Staining intensity was noted to be slightly weaker in Labs 101, 111, 114 and 144 compared to most other labs, but still satisfactory. In particular for Labs 164 and 186, cIQc assessors reported having more difficulty with interpretation of staining as a result of generally weak intensity. Furthermore, for Lab 186, significant background staining of endothelial cells was noted, interfering with interpretation of positive staining in stromal cells and leading to the higher number of "failed" scores reported during self-assessment and independent review. Lastly, Lab 202 was noted to possess generally higher cytoplasmic background staining than other labs, leading to false-positive staining in cores 4, 13 and 15 (corrected in the Garrattogram to the right, which summarizes MLH1 results by cIQc assessors).

Core/Lab	101	102	106	107	111	112	113	114	116	123	125	144	145	149	164	175	186	189	191	200	202	R1
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2	U	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	F	E	E
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
6	U	U	F	F	U	A	U	F	E	U	F	A	F	U	F	E	F	E	U	F	U	A
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	E
8	U	U	U	U	U	E	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	E
9	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
10	U	E	E	U	U	E	U	E	U	U	U	E	E	E	E	U	U	E	E	U	U	E
11	U	U	U	U	U	E	U	F	E	U	U	U	U	U	U	U	U	U	F	U	U	E
12	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
13	A	A	A	A	A	E	A	A	A	A	A	E	A	A	A	E	A	A	A	A	A	A
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A
16	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
18	U	A	U	U	F	A	U	A	U	U	U	F	A	U	U	U	F	U	U	U	U	A
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
20	E	U	U	F	U	E	E	F	E	E	E	E	E	U	U	E	F	U	U	E	E	E
21	U	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E
22	U	U	U	U	U	F	U	F	U	U	U	A	A	U	U	U	U	U	U	U	U	F
23	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
24	U	E	E	U	U	E	E	E	U	U	U	E	E	U	E	U	U	E	U	U	U	E
25	U	A	A	U	A	A	A	F	A	A	A	A	A	A	F	A	F	A	A	F	U	A
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E
27	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	F	E	E	E	E	E
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	U	U	U	E	U	U	U	F	E	E	U	U	U	U	U	E	U	U	U	F	E	E
30	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
31	A	U	U	A	U	A	A	A	A	A	U	U	U	A	U	A	A	A	A	U	A	A

A Absent
E Expressed
F Failed
U Unsatisfactory

MSH2: Cores 8, 11, 18, 20, and 22 were excluded from all statistical analyses because more than 50% of participating laboratories experienced core dropout/staining failure for a single core and reported an unsatisfactory/failed score. An exception was made for the few cores on the tissue microarray that were determined to lack MSH2 expression according to the reference laboratory (i.e. cores 11, 27 and 29) and ANY participating lab was able to report expression or absence of staining.

After cIQc independent review, the majority of participating laboratories were observed to have adequate MSH2 staining. Unusual vascular background staining was observed in Labs 101, 114, 175 and 189, potentially complicating interpretation. Several discordant cores were reported by self-assessment from Lab 164 (left unaltered in the Garrattogram below). cIQc assessors observed that staining in Lab 164 was very weak overall and possessed significant general background that was particularly strong in vascular elements. As a result, interpretation of MSH2 staining by Lab 164 was understandably challenging since positive tumor expression was often masked by such background staining. For Lab 191, general overstaining was noted, leading to a false-positive score for core 27 after cIQc independent review (NOTE: original self-assessment by Lab 191 reported the core as "A", which has been corrected in the Garrattogram below that summarizes results after cIQc independent review for all labs except Lab164.)

Core/Lab	101	102	106	107	111	112	113	114	116	123	125	144	145	149	164	175	186	189	191	200	202	R1
1	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
2	U	E	E	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E
4	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
6	E	E	E	U	E	E	U	E	E	E	E	E	E	U	U	E	U	E	E	E	U	E
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E
8	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	E
9	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
10	U	E	U	U	E	E	U	E	E	E	E	E	E	E	E	U	E	U	U	U	U	E
11	U	U	U	U	U	U	U	F	U	U	U	U	U	U	U	U	U	U	U	U	U	A
12	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	E	E	E
13	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E
15	E	E	E	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E
16	E	E	E	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
18	U	E	U	U	E	E	U	U	E	E	E	E	E	E	F	U	E	U	U	U	U	E
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
20	E	U	E	U	E	E	E	U	U	U	U	E	U	U	U	U	U	U	U	U	A	E
21	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E
22	U	U	U	U	E	U	U	U	U	E	U	E	E	U	U	U	U	U	U	U	U	E
23	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
24	U	E	E	U	E	E	U	E	E	E	E	E	E	E	U	E	E	E	U	U	U	E
25	U	E	E	F	F	E	E	F	U	F	F	E	E	E	F	E	E	E	F	F	U	E
26	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	U	E	E	E
27	A	U	A	F	U	A	A	F	U	U	U	A	U	U	U	U	U	A	E	U	U	A
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	U	U	U	U	U	U	U	U	F	U	E	U	U	U	U	U	U	U	U	U	U	A
30	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
31	E	E	E	U	E	U	E	E	E	E	E	U	E	U	U	E	E	E	U	U	E	E

A Absent
E Expressed
F Failed
U Unsatisfactory

MSH6: Cores 8, 11, 20, and 22 were excluded from all statistical analyses because more than 50% of participating laboratories experienced core dropout/staining failure for a single core and reported an unsatisfactory/failed score. Once again, an exception was made if the core was one of the few cores on the tissue microarray that were determined to lack MSH6 expression according to the reference laboratory (i.e. cores 11, 27 and 29) and ANY participating lab was able to report expression or absence of staining.

Similar to analyses above, the majority of participating laboratories were observed to have adequate staining for this marker. Slightly weaker MSH6 staining compared to other labs was observed in Labs 114 and 191, but still satisfactory. Generally very weak staining was observed from Labs 144 and 164, leading to false-negative or failed cores based on both self-assessment and cIQc independent review. Lab 107 was observed to have slight cytoplasmic background staining overall, while relatively intense cytoplasmic background staining was observed in Lab 189. In neither case did the cytoplasmic background staining appear to interfere with correct interpretation of staining, but overall interpretation was generally more difficult.

Core/Lab	101	102	106	107	111	112	113	114	116	123	125	144	145	149	164	175	186	189	191	200	202	R1
1	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
2	U	E	E	E	E	E	E	E	E	E	E	A	E	U	F	E	E	E	U	E	E	E
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E
4	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
6	U	E	E	E	U	E	E	E	E	E	E	E	U	E	F	E	U	E	U	E	U	E
7	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
8	U	U	E	E	U	E	U	U	U	U	E	U	U	U	U	E	U	U	U	U	U	E
9	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
10	U	E	E	E	U	E	E	E	E	E	E	U	U	E	E	E	U	E	U	U	U	E
11	U	U	U	U	U	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	A
12	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
13	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
15	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E
16	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
18	U	E	E	E	U	E	E	E	E	E	E	U	U	E	E	E	U	U	U	U	U	E
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
20	E	U	E	U	E	E	U	U	U	E	U	U	E	E	U	E	U	U	E	U	E	E
21	U	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	U	E	E	E
22	U	U	E	E	U	E	E	U	U	F	E	U	U	U	F	E	U	U	U	U	U	E
23	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
24	U	E	E	E	U	E	E	E	E	E	E	U	U	E	E	E	U	E	U	E	U	E
25	U	E	E	F	E	E	E	F	E	E	F	F	E	E	A	E	F	E	F	A	E	E
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
27	A	A	A	F	A	U	A	F	A	U	U	A	A	U	U	U	U	A	F	U	U	A
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	U	U	U	U	A	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	A
30	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
31	E	E	E	U	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E

A Absent
E Expressed
F Failed
U Unsatisfactory

PMS2: Cores 6, 8, 11, 20, 22, and 29 were excluded from all statistical analyses for two possible reasons: 1) a core was determined to have a highly heterogeneous tumor content across participating labs upon independent review or 2) more than 50% of participating laboratories experienced core dropout/staining failure for a single core and reported an unsatisfactory/failed score.

Independent review of available PMS2 slides confirmed that no discordant cores were observed in any cores lacking expression of PMS2 according to the reference laboratory. Slightly weaker staining compared to other labs was observed in Labs 116, 144, 145 and 189 (particularly the benign cells), but did not interfere with correct interpretation of staining. Due to considerable overstaining, all cores were reassessed for Lab 202. Despite the apparent overstaining and high background, results from cIQc assessors were identical to the original self-assessments submitted.

Core/Lab	101	102	106	107	111	112	113	114	116	123	125	144	145	149	164	175	186	189	191	200	202	R1
1	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	F	E	E	E
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	F	E	E	E
6	U	F	F	F	U	F	A	F	A	U	F	A	F	A	F	E	F	F	U	F	U	F
7	A	A	A	A	A	A	A	U	A	A	A	A	A	A	F	A	A	A	U	A	A	A
8	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	E
9	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
10	U	U	E	E	E	E	E	U	E	U	U	E	E	E	U	U	U	E	U	E	U	E
11	U	U	U	U	U	U	U	F	U	U	U	U	U	U	U	U	U	U	U	U	U	E
12	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E
13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
16	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
18	U	U	A	A	F	U	A	F	A	U	U	A	A	A	U	U	U	U	U	A	U	A
19	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
20	E	U	F	U	U	U	U	E	E	U	E	E	E	U	F	U	E	U	F	E	U	E
21	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E
22	U	U	U	U	U	U	U	U	A	U	U	U	F	U	U	U	U	U	U	F	U	F
23	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
24	U	U	E	E	E	E	E	U	E	E	U	E	E	E	U	U	U	E	U	E	U	E
25	U	A	A	A	A	F	A	F	A	A	F	A	A	A	F	A	A	A	U	F	A	A
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
27	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	U	U	E
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	U	U	U	U	U	U	U	E	E	U	A	U	U	U	U	U	U	U	U	F	U	E
30	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
31	A	A	U	U	U	A	U	A	A	A	U	U	A	U	U	A	A	A	A	U	A	A

A Absent
E Expressed
F Failed
U Unsatisfactory

Overall Run 31 Conclusions

In general, the 2013 MMR enzyme proficiency testing challenge has provided further encouraging results for MMR enzyme staining in diagnostic pathology laboratories. The sensitivity of MMR-IHC continues to be extraordinarily high and confirms the robustness of the test for use in the universal screening of newly diagnosed colorectal, endometrial and non-serous ovarian cancer for Lynch Syndrome interpretation. Please note that Supplementary Tables 1 to 4 summarizing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen's kappa for each participating laboratory can be found at the end of this document. Due to high dropout of cores lacking expression of MSH2 and MSH6, specificities could not be calculated for a majority of laboratories. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation in mind. Particular attention should be paid to false-positive staining of MMR enzymes due to either background or generally weak staining that may complicate interpretation. For your reference, Tables S5 to S8 provide a summary of the staining protocols used in each participating laboratory.

Your regular participation in cIQc is greatly appreciated and we look forward to working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes in the future as we continue to improve our external quality assurance services.

Table S1. MLH1 descriptive statistics based on corrected self-assessments

Lab	Total N	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	24	83.33	20	20/20 (100%)	1	1	1	1	1
102	24	91.67	22	22/22 (100%)	1	1	1	1	1
106	24	91.67	22	22/22 (100%)	1	1	1	1	1
107	24	83.33	20	20/20 (100%)	1	1	1	1	1
111	24	87.5	21	21/21 (100%)	1	1	1	1	1
112	24	100	24	23/24 (96%)	1	0.83	0.95	1	0.88
113	24	95.83	23	23/23 (100%)	1	1	1	1	1
114	24	91.67	22	22/22 (100%)	1	1	1	1	1
116	24	95.83	23	23/23 (100%)	1	1	1	1	1
123	24	95.83	23	23/23 (100%)	1	1	1	1	1
125	24	87.5	21	21/21 (100%)	1	1	1	1	1
144	24	95.83	23	22/23 (96%)	1	0.8	0.95	1	0.86
145	24	95.83	23	23/23 (100%)	1	1	1	1	1
149	24	91.67	22	22/22 (100%)	1	1	1	1	1
164	24	83.33	20	20/20 (100%)	1	1	1	1	1
175	24	91.67	22	21/22 (95%)	1	0.83	0.94	1	0.88
186	24	75	18	18/18 (100%)	1	1	1	1	1
189	24	95.83	23	23/23 (100%)	1	1	1	1	1
191	24	95.83	23	23/23 (100%)	1	1	1	1	1
200	24	83.33	20	20/20 (100%)	1	1	1	1	1
202	24	87.5	21	21/21 (100%)	1	1	1	1	1

Table S2. MSH2 descriptive statistics based on corrected self-assessments

Lab	Total N	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	26	76.92	20	20/20 (100%)	1	1	1	1	1
102	26	92.31	24	24/24 (100%)	1	--	1	--	--
106	26	92.31	24	24/24 (100%)	1	1	1	1	1
107	26	73.08	19	19/19 (100%)	1	--	1	--	--
111	26	88.46	23	23/23 (100%)	1	--	1	--	--
112	26	92.31	24	24/24 (100%)	1	1	1	1	1
113	26	84.62	22	22/22 (100%)	1	1	1	1	1
114	26	88.46	23	23/23 (100%)	1	--	1	--	--
116	26	88.46	23	23/23 (100%)	1	--	1	--	--
123	26	88.46	23	23/23 (100%)	1	--	1	--	--
125	26	88.46	23	22/23 (96%)	1	0	0.96	--	0
144	26	88.46	23	23/23 (100%)	1	1	1	1	1
145	26	92.31	24	24/24 (100%)	1	--	1	--	--
149	26	80.77	21	21/21 (100%)	1	--	1	--	--
164	26	65.38	17	14/17 (82%)	0.82	--	1	0	0
175	26	88.46	23	23/23 (100%)	1	--	1	--	--
186	26	88.46	23	23/23 (100%)	1	--	1	--	--
189	26	92.31	24	24/24 (100%)	1	1	1	1	1
191	26	76.92	20	19/20 (95%)	1	0	0.95	--	0
200	26	76.92	20	20/20 (100%)	1	--	1	--	--
202	26	69.23	18	18/18 (100%)	1	--	1	--	--

Table S3. MSH6 descriptive statistics based on corrected self-assessments

Lab	Total N	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	27	66.67	18	18/18 (100%)	1	1	1	1	1
102	27	96.3	26	26/26 (100%)	1	1	1	1	1
106	27	96.3	26	26/26 (100%)	1	1	1	1	1
107	27	85.19	23	23/23 (100%)	1	--	1	--	--
111	27	85.19	23	23/23 (100%)	1	1	1	1	1
112	27	88.89	24	24/24 (100%)	1	--	1	--	--
113	27	92.59	25	25/25 (100%)	1	1	1	1	1
114	27	88.89	24	24/24 (100%)	1	--	1	--	--
116	27	96.3	26	26/26 (100%)	1	1	1	1	1
123	27	92.59	25	25/25 (100%)	1	--	1	--	--
125	27	88.89	24	24/24 (100%)	1	--	1	--	--
144	27	81.48	22	21/22 (95%)	0.95	1	1	0.5	0.65
145	27	81.48	22	22/22 (100%)	1	1	1	1	1
149	27	88.89	24	24/24 (100%)	1	--	1	--	--
164	27	70.37	19	18/19 (95%)	0.95	--	1	0	0
175	27	92.59	25	25/25 (100%)	1	--	1	--	--
186	27	74.07	20	20/20 (100%)	1	--	1	--	--
189	27	92.59	25	25/25 (100%)	1	1	1	1	1
191	27	66.67	18	18/18 (100%)	1	--	1	--	--
200	27	85.19	23	22/23 (96%)	0.96	--	1	0	0
202	27	77.78	21	21/21 (100%)	1	--	1	--	--

Table S4. PMS2 descriptive statistics based on corrected self-assessments

Lab	Total N	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	25	80	20	20/20 (100%)	1	1	1	1	1
102	25	88	22	22/22 (100%)	1	1	1	1	1
106	25	96	24	24/24 (100%)	1	1	1	1	1
107	25	92	23	23/23 (100%)	1	1	1	1	1
111	25	92	23	23/23 (100%)	1	1	1	1	1
112	25	88	22	22/22 (100%)	1	1	1	1	1
113	25	96	24	24/24 (100%)	1	1	1	1	1
114	25	80	20	20/20 (100%)	1	1	1	1	1
116	25	100	25	25/25 (100%)	1	1	1	1	1
123	25	92	23	23/23 (100%)	1	1	1	1	1
125	25	76	19	19/19 (100%)	1	1	1	1	1
144	25	96	24	24/24 (100%)	1	1	1	1	1
145	25	92	23	23/23 (100%)	1	1	1	1	1
149	25	92	23	23/23 (100%)	1	1	1	1	1
164	25	72	18	18/18 (100%)	1	1	1	1	1
175	25	88	22	22/22 (100%)	1	1	1	1	1
186	25	88	22	22/22 (100%)	1	1	1	1	1
189	25	96	24	24/24 (100%)	1	1	1	1	1
191	25	68	17	17/17 (100%)	1	1	1	1	1
200	25	88	22	22/22 (100%)	1	1	1	1	1
202	25	84	21	21/21 (100%)	1	1	1	1	1

Table S5. MLH1 staining protocols

Lab	Clone	Dilution	Supplier	Ag Retrieval	Detection	Enhancement	Chromagen
101	ESO5	1:50	Leica	CC1	OptiView	Cu	DAB
102	ES05	1/40	LEICA	FLEX HIGH PH TRS	FLEX	CUSO4	DAB+
106	ES05	1:150	Leica	T-Hcl ph9.0, microwave pressure	Biocare Polymer	none	DAB
107	ES05	1:25, 32 minutes	Novocastra	CCI - standard	Ventana Ultraview	Ventana Amplification	DAB
111	G168-15	1/20	BD PHAMINGEN	CC1 64 MINUTES	VENTANA ULTRAVIEW	AMPLIFIER AND COPPER	DAB
112	M1	RTU	VENTANA	CC1 56 minutes	OPTIVIEW	Copper	DAB
113	ES05	PD	DAKO	High pH buffer	DAKO Flex +	n/a	DAB
114	ES05	1/50	Leica	CC1-32min	Ventana Optiview	Copper	DAB
116	G168-15	1/40	BD Pharmingen	CC1 64min	Optiview DAB Ventana	no	DAB
125	ES05	1/100	Dako	ER2-20	Bond Polymer Refine	Bond Enhancer	DAB
144	M1	Predilute	Ventana	CC1, 56 minutes	Optiview	Copper	DAB
145	g168-728	1/10 from concentrate	cell marque	CC1 60 mins	XT ultraView DAB v3	yes, ventana	DAB
149	ES05	RTU	Dako IR079	PT Link high pH 96 C 30 min	Envision Flex	No	DAB
164	G168-728	predilute	Ventana	ultra CC1	Optiview	no	DAB
175	9168-728	Predilute	Ventana	Yes	Ultraview	No	DAB
186	G168-728	1:50	Cell Marque	EDTA buffer, 20'	Bond refine detection kit	None	DAB
189	M1	Predilute	Ventana	Cell Conditioning I	OptiView DAB	Copper	DAB
191	E505	1/10	novocastra	CC1	ultra view roche	amlificatie	DAB
200	G168-728	RTU	Cell Marque	EDTA	ultraView	N/A	DAB
202	ES05	1:10	BD PHARMINGEN	ER2(20)	REFINE DETECTION LEICA KIT	NONE	DAB
R1	G168-728	1/25	Cell Marque	CC1 32mins	OptiView	Copper	DAB

Table S6. MSH2 staining protocols

Lab	Clone	Dilution	Supplier	Ag Retrieval	Detection	Enhancement	Chromagen
101	G219-1129	1:1000	Cell marque	CC1	OptiView	Cu	DAB
102	FE11	1/50	CALBIOCHEM	FLEX HIGH PH TRS	FLEX	CUSO4	DAB+
106	25D12	1:75	Leica	T-Hcl pH9.0, microwave pressure	Biocare Polymer	none	DAB
107	25D12	1:5, 1 hour	Novocastra	CCI - standard	Ventana Ultraview	Ventana Amplification	DAB
111	G219-1129	1/400	BD PHARMINGEN	CC1 64 MINUTES	VENTANA IVIEW	COPPER	DAB
112	G219-1129	RTU	VENTANA	CC1 56 minutes	OPTIVIEW	Copper	DAB
113	25D12	1/25	Leica	High pH buffer	DAKO Flex +	n/a	DAB
114	G219-1129	1/1000	Cell Marque	CC!-32min	Ventana Optiview	Copper	DAB
116	G219-1129	1/1600	Cell Marque	CC1 -32min	Optiview DAB	no	DAB
125	25D12	predil	Novocastra	ER2-20	Bond Polymer Refine	Bond Enhancer	DAB
144	G219-1129	Predilute	Cell Marque	CC11, 16 minutes	Optiview	Copper	DAB
145	G219-1129	PRE-DILUTED	CELL MARQUE	CC1 30 mins	XT ultraView DAB v3	yes, ventana	DAB
149	FE11	RTU	Dako IR085	PT Link high pH 96 C 20 min	Envision Flex Plus	Yes (Linker 15 min)	DAB
164	G219-1129	predilute	Ventana	ultra CC1	Optiview	no	DAB
175	9168-728	Predilute	Ventana	Yes	iView	No	DAB
186	G219-1129	1:100	Cell Marque	EDTA buffer, 20'	Bond refine detection kit	None	DAB
189	G219-1129	Predilute	Ventana Medical Systems	Cell Conditioning I	OptiView	Copper	DAB
191	FE11	1/25	calbiochem	CC1	ultra view roche	amlificatie	DAB
200	G219-1129	RTU	Cell Marque	EDTA	ultraView	N/A	DAB
202	25D12	RTU	LEICA	ER2(30)	REFINE DETECTION LEICA KIT	NONE	DAB
R1	G219-1129	1/1000	Cell Marque	CC1 32 mins	OptiView	Copper	DAB

Table S7. MSH6 staining protocols

Lab	Clone	Dilution	Supplier	Ag Retrieval	Detection	Enhancement	Chromagen
101	EP49	1:200	Epitomics	CC1	OptiView	Cu	DAB
102	EP49	1/100	DAKO	FLEX HIGH PH TRS	FLEX	CUSO4	DAB+
106	SP93	1:50	Cell Marque	T-Hcl pH9.0, microwave pressure	Biocare Polymer	none	DAB
107	44/MSH6	1:300, 32 min	BD	CCI - standard	Ventana Ultraview	None	DAB
111	BC/44	PREDILUTE	BIOCARE MEDICAL	CC1 64 MINUTES	VENTANA ULTRAVIEW	COPPER	DAB
112	EP49	1:750	EPITOMICS	CC1 56 minutes	OPTIVIEW	copper	DAB
113	EP49	PD	DAKO	High pH buffer	DAKO Flex	n/a	DAB
114	EP49	1/200	Epitomics	CC!-32min	Ventana Optiview	Copper	DAB
116	BC/44	1/600	Biocare Medical	CC1 32min	Optiview DAB Ventana	no	DAB
125	44/MSH6	1/2000	BD Biosciences	ER2-20	Bond Polymer Refine	Bond Enhancer	DAB
144	44	Predilute	Ventana	CC1, 16 minutes	Optiview	Copper	DAB
145	44	PRE- DILUTED	cell marque	CC1 60 mins	XT ultraView DAB v3	yes, ventana	DAB
149	EP49	RTU	Dako IR086	PT Link high pH 96 C 20 min	Envision Flex	No	DAB
164	44	predilute	Ventana	ultra CC1	Optiview	no	DAB
175	44	1/100	Cell Marque	Yes	iView	No	DAB
186	BC/44	1:50	Biocare Medical	Citrate buffer, 20'	Bond refine detection kit	None	DAB
189	44	predilute	Ventana	Cell Conditioner I	OptiView DAB	Copper	DAB
191	EPR3947	RTU	Roche	CC1	ultra view roche	amlificatie	DAB
200	44	RTU	Cell Marque	EDTA	ultraView	N/A	DAB
202	PU29	1:25	CEDARLANE	ER2 HIGH PH 40 MIN	REFINE POLYMER DETECTION LEICA	NONE	DAB
R1	EP49	1/200	Epitomics	CC1 32 mins	OptiView	Copper	DAB

Table S8. PMS2 staining protocols

Lab	Clone	Dilution	Supplier	Ag Retrieval	Detection	Enhancement	Chromagen
101	EP51	1:15	Epitomics	CC1	OptiView	Cu	DAB
102	A16-4	1/20	BD PHARMINGEN	FLEX HIGH PH TRS	FLEX	CUSO4	DAB+
106	MRQ-28	1:75	Cell Marque	T-Hcl pH9.0, microwave pressure	Biocare Polymer	none	DAB
107	A16-4	1:100, 30 minutes	BD	Decloak Flex TRS High pH 9.0	Flex + 30 (DAKO)	None	DAB
111	EPR 3947	PREDILUTE	VENTANA	CC1 36 MINUTES	VENTANA ULTRAVIEW	AMPLIFIER AND COPPER	DAB
112	EP51	1:20	EPITOMICS	CC1 56 minutes	OPTIVIEW	Copper	DAB
113	PMS2	1/20	BD	High pH Buffer	DAKO Flex +	n/a	DAB
114	EP51	1/25	Epitomics	CC!-32min	Ventana Optiview	Copper	DAB
116	MRQ-28	1/10	Cell Marque	CC1 64min	Optiview DAB	no	DAB
125	A16-4	1/100	BD Biosciences	ER2-20	Bond Polymer Refine	Bons Enhancer	DAB
144	EPR3947	Predilute	Cell Marque	CC1, 64 minutes	Optiview	Copper	DAB
145	MRQ-28	1/5 FROM CONCENTRATED	CELL MARQUE	CC1 60 mins	XT ultraView DAB v3	yes, ventana	DAB
149	EP51	RTU	Dako IR087	PT Link high pH 96 C 20 min	Envision Flex Plus	Yes (Linker 15 min)	DAB
164	EPR3947	predilute	Ventana	ultra CC1	Optiview	no	DAB
175	EPR394	Predilute	Ventana	Yes	Ultraview	Amplifier	DAB
186	ERP3947	1:4	CELL MARQUE	EDTA BUFFER, 20'	Bond refine detection kit	None	DAB
189	EPR3947	Predilute	Ventana Medical Systems	Cell Conditioning I	OptiView	Copper	DAB
191	44	1/800	BD transduction laboratories	CC1	ultra view roche	amlificatie	DAB
200	EPR3947	RTU	Cell Marque	EDTA	ultraView	N/A	DAB
202	A16-4	1:10	BD PHARMINGEN	ER2(30)	REFINE DETECTION LEICA KIT	NONE	DAB
R1	A16-4	1/20	Biocare	CC1 32 mins	OptiView	Copper	DAB