Assessors' report for cIQc Run 66: MMR Immunohistochemistry

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Assessment performed on Monday, November 28th, 2016 at Royal Victoria Regional Health Centre, Barrie ON

Overview

A total of 31 labs participated in Run 66. Slides from Labs 114, 193 and 202 were not returned in time for the cIQc assessment meeting. Available slides from all other participating labs were reviewed by cIQc assessors who had details about staining protocols but were blinded to lab identity. Independent review led to alteration of original self-reported results for discordant cores; statistics were calculated based on the review assessment, as we believe this more accurately reflects the technical performance (but note that it does not reflect interpretive performance, which is not formally assessed through CIQC). 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; this is particularly true for large specimens e.g. colectomy or hysterectomy, where there has been delayed fixation, as MMR proteins are very fixation sensitive and degrade rapidly. In general, interpretation is easier based on biopsy specimens, as they are promptly fixed.

MMR immunohistochemical staining continues to be of very good quality. All labs were considered to have results that were uniformly technically interpretable/optimal by cIQc assessors, with the exceptions noted below.

Although the technical quality of MMR staining is very good (and dramatically improved from years past), the biology of MMR protein expression continues to cause problems. This is especially true for tumours with methylation of the promoter of MLH1, which can show complete loss of MLH1 and PMS2 expression, or patchy expression of either protein, and may even show patchy loss of MSH6 (as a secondary event, due to a hypermutable region in exon 5 of MSH6 that can become mutated as a result of MLH1 loss).

Most technical issues related to use of ready to use/predilute antibodies, or use of an amplification/enhancement step. For some labs this resulted in increased background, as noted below. Amplification can be highly effective, but in our experience protocols with amplification show more run to run variability than protocols without amplification, so amplification should be avoided if possible. Where it must be used, consideration should be given to use of on slide controls, in order to be able to assess run to run variation. On slide controls are not routinely used by all labs for MMR immunostaining as there is invariably internal positive control staining, but with concerns about false positive staining when there is amplification of signal, an on slide control consisting of a case with loss of expression of the protein of interest could be of value.

General observations by assessors for each marker of the MMR panel are detailed below.

Definitions

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 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.

MLH1: MLH1 staining results were, overall, excellent. As expected, there was heterogeneity of MLH1 expression associated with MLH1 promoter methylation. For cores 1, 13, 24, 39 and 40 there was heterogeneity of expression reflected in variable staining results, so the cores were excluded from assessment. Lab 175 had some weak nuclear punctate staining in a few cores (e.g. core 24), which they ignored, correctly. This pattern of staining was also seen in the slide from Lab 222 (e.g. core 33). It is distinct from the uniform nuclear staining of true positive staining and we are uncertain of the cause of this pattern of staining, but those labs should be aware of this pattern of presumably artifactual staining. The Garrattogram after cIQc assessment of MLH1 is shown below.

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	193	202	217	220	222	**MMR status**
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH6
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2
4	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
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	U	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	PMS2
7	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
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	U	A	A	U	U	A	A	A	F	F	A	U	U	A	U	A	A	A	E	U	U	E	A	U	E	A	U	A	MLH1
10	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	F	A	A	E	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	U	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	U	U	E	E	E	E	E	E	E	E	E	U	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	E	E	E	E	E	E	E	MSH6
15	E	E	E	E	E	E	F	E	E	E	F	E	F	E	E	E	E	E	E	E	E	E	E	E	E	F	F	E	E	E	E	MSH6
16	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	MLH1
17	U	U	A	A	A	A	U	A	A	A	U	A	U	A	A	A	A	A	A	A	A	A	U	A	A	A	U	A	A	A	A	MLH1
18	E	E	U	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	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	A	A	A	F	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	A	A	A	A	F	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	F	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	F	A	A	A	MLH1
23	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	F	E	E	E	E	MSH2
25	U	U	A	A	U	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	PMS2
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	A	A	F	A	A	A	A	MLH1
27	F	A	A	A	A	A	F	F	A	A	A	F	A	A	F	A	A	F	A	A	A	A	A	A	A	A	F	A	A	A	A	MLH1
31	E	E	E	E	E	E	E	E	E	U	A	U	A	E	E	E	U	E	E	E	E	U	A	U	U	A	A	U	E	U	E	PMS2
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	A	A	A	A	F	A	A	A	A	A	E	E	A	A	A	A	A	A	A	A	U	A	E	A	U	E	E	A	E	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	MLH1
35	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	MSH6
37	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	U	U	F	U	A	A	MLH1
38	E	E	E	E	E	E	F	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	F	F	E	E	E	E	E	MSH2

PMS2: PMS2 staining results were also very good, overall. Labs 103 and 109 had high nuclear background staining, that we attribute to the amplification step. This effect can show run to run variability i.e. present one day and not another, but it is important to be aware of it. As noted previously, appropriate on slide controls can show if there is increased background staining in cells with loss of expression. Labs 115 and 217 had high cytoplasmic background staining that we attribute to use of a RTU/predilute antibody; results were nonetheless interpretable. Labs 189 and 222 had **suboptimal** results, with high background/false positive staining results; both labs were using RTU/predilute Abs. The Garrattogram after cIQc assessment of PMS2 is shown below.

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	193	202	217	220	222	**MMR status**
1	F	E	E	E	E	E	U	U	E	E	E	E	U	E	E	U	U	U	U	U	U	U	U	U	U	E	U	U	E	U	U	MLH1
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	MSH6
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH2
4	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	F	A	A	A	U	MLH1
5	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	F	A	A	A	U	PMS2
6	A	A	U	A	A	A	A	A	A	A	A	U	A	A	A	F	A	A	A	A	A	A	A	U	A	E	A	A	A	A	U	PMS2
7	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	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	E	E	E	E	E	E	U	MSH2
9	A	U	U	A	A	F	U	U	A	A	A	F	A	A	A	U	A	U	A	A	A	U	U	U	E	U	A	U	A	U	E	MLH1
10	A	A	F	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	F	A	A	A	U	MLH1
11	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	E	MSH2
12	A	A	U	F	A	A	A	F	A	A	A	A	F	A	A	U	A	A	A	A	A	A	A	A	A	E	F	A	A	A	U	PMS2
13	F	U	U	E	E	U	U	U	U	U	U	U	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	E	U	U	U	MLH1
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MSH6
15	E	E	E	E	E	E	E	E	E	E	F	E	F	E	E	E	E	E	E	E	E	E	F	E	E	F	F	E	E	F	U	MSH6
16	A	A	U	A	A	A	U	A	A	A	U	U	A	U	U	A	A	U	A	A	A	A	A	U	A	U	A	A	U	A	U	MLH1
17	U	A	U	U	A	A	U	A	A	A	U	U	A	U	U	A	A	U	A	A	A	A	A	U	A	U	U	A	U	A	U	MLH1
18	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	U	E	E	E	MSH6
19	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	U	MLH1
20	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	E	A	A	A	A	A	F	A	A	A	U	MLH1
21	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	MLH1
22	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	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	F	E	E	E	U	MSH2
24	A	A	U	A	F	E	A	E	A	E	A	A	A	A	A	A	A	A	E	E	E	U	E	A	E	A	U	U	A	A	U	MLH1
25	U	A	E	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	A	A	U	PMS2
26	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	U	MLH1
27	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	F	A	A	A	U	MLH1
32	A	A	U	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	MLH1
33	A	A	U	A	A	A	U	A	A	A	A	A	A	A	A	F	A	U	A	A	A	A	A	U	A	U	A	A	U	A	U	PMS2
34	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	MLH1
35	U	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	U	E	E	E	E	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	E	F	E	E	E	E	E	MSH2

MSH2: MSH2 staining results were excellent. Labs 116 and 186 had weaker staining (adequate), relative to other labs, but results were interpretable. Lab 123 had perfect staining, from an esthetic perspective, and was awarded a gold star by the assessors! The Garrattogram after cIQc assessment of MSH2 is shown below.

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	193	202	217	220	222	**MMR status**
2	E	E	E	E	E	E	F	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	U	F	E	E	E	E	E	MSH6
3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MSH2
4	E	E	E	E	E	F	E	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	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	PMS2
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
8	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	F	F	A	A	A	A	MSH2
9	E	E	E	E	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	U	E	E	E	E	U	E	MLH1
10	E	E	E	E	E	U	F	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	U	E	E	F	F	F	E	E	E	MLH1
11	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	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	U	E	E	E	E	E	PMS2
14	E	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	MSH6
16	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	U	E	E	E	E	MLH1
17	E	E	U	E	E	U	E	E	E	U	E	U	E	E	E	E	E	E	E	E	U	E	E	U	U	E	U	U	U	E	E	MLH1
18	E	U	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	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	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	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	E	F	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	MLH1
23	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	F	F	F	A	A	A	A	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	MLH1
25	E	E	U	U	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
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
27	E	E	E	E	E	E	F	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	F	F	F	F	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	E	E	E	E	E	E	E	MLH1
33	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	U	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	MLH1
35	F	E	U	U	E	E	F	E	E	E	E	U	F	E	E	E	E	E	E	E	E	E	E	E	F	F	F	E	E	E	E	MSH6
37	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	F	E	U	E	E	E	U	MLH1
38	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	F	A	A	A	A	A	MSH2
40	E	E	E	E	E	F	E	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	U	E	E	E	U	E	E	E	E	MLH1

MSH6: MSH6 staining continues to present challenges. As noted in previous run summaries, weak MSH6 weak staining can be seen with MSH2 loss e.g. cores 2 and 23**. Please continue to take into account the possibility that very weak MSH6 expression may be associated with absence of expression of MSH2**, especially if you are using two markers (PMS2 and MSH6) rather than four, for assessment of MMR status. Please note that calculation of individual lab clinical test sensitivity and specificity for MSH6 is particularly problematic because of the cores with weak/equivocal staining and uncertainty for those cores about what the "reference" value should be. Lab 107 had cytoplasmic background staining (perhaps attributable to the 1:20 primary antibody dilution). Lab 189 had weak overall staining and this resulted in many failed results. Lab 222 had high granular background, attributable to amplification/enhancement. The Garrattogram after cIQc assessment of MSH6 is shown below.

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	193	202	217	220	222	**MMR status**
2	A	A	A	A	A	F	A	A	A	F	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	F	A	A	E	A	A	MSH6
3	E	E	A	F	F	F	A	F	E	A	A	A	E	A	E	E	E	A	A	A	E	A	A	A	E	A	A	E	E	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	F	E	E	E	E	E	MLH1
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	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	E	E	E	E	E	E	E	F	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	F	A	A	A	A	F	F	U	E	A	A	A	MSH2
9	E	E	U	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	U	E	E	E	U	U	E	E	E	E	E	E	E	MLH1
10	E	A	A	A	A	U	A	E	A	F	A	A	A	A	E	A	A	A	A	F	E	E	U	A	A	F	F	U	A	U	E	MLH1
11	A	E	E	F	A	U	A	A	E	A	A	A	E	A	E	E	A	A	A	A	A	A	A	A	A	A	A	E	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	U	E	E	E	E	E	PMS2
14	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	F	A	A	A	A	A	MSH6
16	E	E	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	E	E	E	MLH1
17	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	U	E	U	E	E	U	E	E	E	E	E	E	E	MLH1
18	A	A	A	A	A	U	A	A	A	F	A	A	F	A	A	A	A	A	E	F	A	A	A	A	A	F	A	A	E	A	A	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	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	E	F	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	F	E	E	E	E	E	F	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	MLH1
23	A	E	A	F	A	F	A	A	A	A	A	F	F	A	E	E	A	A	A	F	A	A	A	A	A	F	A	E	A	A	A	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	MLH1
25	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	PMS2
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	MLH1
27	A	A	A	A	F	F	F	A	U	F	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	F	F	A	A	A	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	E	F	E	E	E	E	E	MLH1
33	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	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	F	E	E	E	E	E	MLH1
35	E	E	F	E	U	U	F	E	E	F	E	F	A	F	E	E	E	F	A	F	E	E	A	F	A	F	F	E	U	F	A	MSH6
37	U	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	U	U	E	E	E	U	E	E	U	U	MLH1
38	A	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	F	A	A	A	A	A	F	A	A	A	A	A	MSH2
40	E	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	U	E	E	F	E	E	E	E	E	MLH1

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 this reservation. 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	Antigen Retrieval Method	Antigen Retrieval Time	Antibody Clone	Antibody Dilution	Antibody Incubation Time	Antibody Supplier	Antibody Lot #	Detection System	Amplification Y/N	Enhancement Y/N	Chromogen
101	CC1	64min	ES05	1:50	32min@37	Leica	6025230	Optiview	n	n	DAB
102	DAKO PT HIGH pH	10/20/10	ES05	1:80	30"RT	DAKO	10105399	DAKO FLEX+	YES	CUSO4	DAB+
103	CC1	56 MINS	M1	PRE	32 MINS	VETNANA	F02382	OPTIVEIW	n	y	dab
104	HIER (heat induced epitope retrieval)	20 min	ES05	RTU	30 min	DAKO	10101385	polymere	yes, mouse	non	DAB
106	microwave pressure cooker	30 min	ES05	1:85	45 minutes	Novacastra	6039695	MACH4	no	no	DAB
107	Ultra cc1	64	ES05	1:20	40 min	Leica/Novocastra	6038153	Optiview DAB	N	Y	DAB
109	HIER CC1 HIGH pH	32 MIN	M1	RTU	16 MIN	VENTANA	F10114	OPTIVIEW	N	Y	DAB
110	Dako PT high ph	20 min @97C	ES05	1:100	20 min	DAKO	10107954	Dako Envision Flex	Y	N	DAB
111	CC1	48 MINUTES	G168-15	1/50	32 MINUTES	BIOCARE	42616	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2	25 minutes	ES05	1:100	30 minutes	Leica (Novocastra)	6023826	BOND Polymer Refine	none	none	DAB
114	CC1	32mins	ES05	1:50	44mins	Leica	6023826	OPTIVIEW	N	N	DAB
115	envision flex high pH	30 mins	ES05	RTU	20 mins	Dako	10115068	envision flex	N	N	DAB
116	CC1	48 MIN	G168-15	1/80	44 MIN	BD PHARMINGEN	2138711	OPTIVIEW DAB IHC	N	COPPER	DAB
123	FLEX HIGH pH	15 MIN	ES05	PREDILUTE	20 MIN	DAKO	10115068	FLEX DAB	Y	N	DAB
124	CC1	64 min	ES05	1/100	16	Leica	6025230	Optiview	non	non	dab
125	HIGH pH	30 min	ES05	predilute	30 MIN	Dako	10103827	ENVISION FLEX	N	N	DAB
126	Microwave Pressure Cooker, citrate buffer ph 6.0	34 minutes	ESO5	1:50	30 MINUTES	DAKO	10115966	ULTRAVISION QUANTO DETECTION SYSTEM, HRP	YES	NO	DAB
138	HIER - EDTA	20	ES05	RTU	20	Dako	10111636	Polymer	Y	N	DAB
141	HIER	20	ES05	1:50	20	Dako	10105399	Polymer	Y	N	DAB
144	CC1	56 min	ES05	1:25	32 min	Novocastra	6039695	Optiview	N	Yes, Copper	DAB
145	CC1	56	G168-15	1/50	32	BIOCARE	20215	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min	ES05	RTU	20 min	Dako	10107799	EnVision Flex	No	No	DAB
175	HIER	64 min	M1	Predilute	16 min	Roche	F10114	Polymer	N	Y	DAB
181	HIER	20 MINUTES	ES05	1:100	20 MINUTES	DAKO	10107954	HRP-POLYMER	N	N	DAB
186	HIER	20 MIN	G168-728	1:50	15 MIN.	CELL MARQUE	1313506H	LEICA BOND POLYMER	N	N	DAB
189	CC1	64	M1	pre-dilute	16	Ventana	unknown	ultraView	N	N	DAB
193	low pH	30 minutes	ES05	RTU	30 minutes	Dako	unknown	Flex +	Yes	No	DAB
202	ER2 Leica	20 min	ES05	1/10	15 min	BD Pharmagen	5329901	Refine Detection kit Leica	no	no	DAB
217	HIER	64	M1	predilute	60	Roche Ventana	G02350	Optiview	N	Y	DAB
220	CC1	72min	ES05	1/25	1h	Dako	10104116	OptiView	N	N	DAB
222	CC1	90min	M1	RTU	4min	Roche	F08049	Optiview DAB	Y	Copper	DAB

Table S2. Reported PMS2 staining protocols.

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time	Antibody Clone	Antibody Dilution	Antibody Incubation Time	Antibody Supplier	Antibody Lot #	Detection System	Amplification Y/N	Enhancement Y/N	Chromogen
101	CC1	32min	EP51	1:15	32min@37	Epitomics	EN051809	Optiview	n	n	DAB
102	DAKO PT HIGH pH	10/20/10	EP51	1:20	30"RT	DAKO	10110537	DAKO FLEX+	YES	CUSO4	DAB+
103	CC1	64 MINS	EPR3947	PRE	1 HOUR	CELL MARQUE	1506813B	OPTIVIEW	YES	Y	DAB
104	HIER (heat induced epitope retrieval)	20 min	EP51	RTU	20 min	DAKO	10107620	polymere	yes, rabbit	no	DAB
106	microwave pressure cooker	30 min	MRQ-28	1:25	45 minutes	Cell Marque	1516709B	MACH4	no	no	DAB
107	Decloaking in Flex TRS High pH 9.0	120C, 30 sec	A16-4	1:200	30 min	BD Pharmingen	5338787	Flex+30	N	N	DAB
109	HIER CC1 HIGH pH	64 MIN	EPR3947	RTU	20 MIN	CELL MARQUE	1523405B	OPTIVIEW	Y	Y	DAB
110	Dako PT high ph	20 min @97C	EP51	1:50	30 MIN	DAKO	10107094	Dako Envision Flex	N	N	DAB
111	CC1	48 MINUTES	EP51	1/100	32 MINUTES	DAKO	10112572	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2	30 minutes	EP51	1;75 using background reducing diluent	30 minutes	DAKO	10109389	BOND Polymer Refine	none	none	DAB
114	CC1	64mins	EP51	1:25	64mins	EPITOMICS	EN 041303	OPTIVIEW	N	N	DAB
115	envision flex high pH	30 mins	EP51	RTU	30 mins	Dako	10111642	envision flex	N	N	DAB
116	CC1	64 MIN	EPR3947	RTU	60 MIN	VENTANA	1532405 B	OPTIVIEW DAB IHC	Y	COPPER	DAB
123	FLEX HIGH pH	15 MIN	A16-4	1/50	30 MIN	BDBIOSCIENCE	5338787	FLEX DAB	Y	N	DAB
124	CC1	56 min	EP51	1/40	32 min	Dako	10108546	optiview	non	non	Dab
125	HIGH pH	30 MIN	EP51	RTU	30 MIN	DAKO	10114394	ENVISION FLEX	N	N	DAB
126	Microwave Pressure Cooker, citrate buffer ph 6.0	34 minutes	EP51	1:25	30 MINUTES	DAKO	10114768	ULTRAVISION QUANTO DETECTION SYSTEM, HRP	YES	NO	DAB
138	HIER - EDTA	20	EP51	RTU	30	Dako	10107620	Polymer	N	N	DAB
141	HIER	20	EP51	1:50	30	Dako	10107094	polymer	Y	N	DAB
144	CC1	64 min	EPR 3947	Pre-Dilute	60 min	Cell Marque	1532405C	Optiview	No	Yes, copper	DAB
145	CC1	64	EPR3947	1/15	44	CELLMARQUE	1506813A	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min	EP51	RTU	20 min	Dako	10108822	EnVison Flex	Yes	No	DAB
175	HIER	64 min	EPR3947	predilute	32 min	Roche	1607510C	polymer	Y	Y	DAB
181	HIER	20 MINUTES	EP51	1:50	30 MINUTES	DAKO	10107094	HRP-POLYMER	N	N	DAB
186	HIER	20 MIN.	ERP3947	1:4	15 MIN.	CELL MARQUE	1506813A	LEICA BOND POLYMER	N	N	DAB
189	CC1	64	EPR3947	pre-dilute	32	Cell Marque	unknown	ultraView DAB	Y	N	DAB
193	high pH	30 minutes	EP51	RTU	30 min	Dako	unknown	Flex +	Yes	No	DAB
202	ER2 Leica	30	A16-4	1/25	15 min	BD Pharmagen	5338787	Leica Define Detection kit	no	no	DAB
217	HIER	92	EPR3947	predilute	96	Roche Ventana	1532405B	Optiview	N	Y	DAB
220	CC1	64min	EP51	1/40	1h8min	DAKO	10108546	OptiView	N	N	DAB
222	CC1	90min	EPR3947	RTU	44min	Roche	1506813E	DAB Optiview	Y	Copper	DAB

Table S3. Reported MSH2 staining protocols.

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time	Antibody Clone	Antibody Dilution	Antibody Incubation Time	Antibody Supplier	Antibody Lot #	Detection System	Amplification Y/N	Enhancement Y/N	Chromogen
101	CC1	32min	G219-1129	1:200	32 min @37	Cell Marque	1313003B	Optiview	n	n	DAB
102	DAKO PT LINK HIGH pH	10/20/10	FE11	1:40	30" RT	DAKO	10106449	DAKO FLEX+	YES	CUSO4	DAB+
103	CC1	56 MINS	G219-1129	PRE	32	CELL MARQUE	1417104F	OPTIVIEW	N	Y	DAB
104	HIER (heat induced epitope retrieval)	20 min	FE11	RTU (ready to use)	30 min	DAKO	10107056	polymere	yes, mouse	no	DAB
106	microwave pressure cooker	30 min	FE11	1:50	45 minutes	Dako	10111672	MACH4	No	No	DAB
107	cc1	32	G219-1129	1:200	32	Cell Marque	1334002A	Optiview DAB	N	Y	DAB
109	HIER CC1 HIGH pH	32 MIN	G219-1129	RTU	8 MIN	CELL MARQUE	1505808F	OPTIVIEW	N	Y	DAB
110	Dako PT high ph	20 min @97C	FE11	1:150	20 min	DAKO	10106449	Dako Envision Flex	Y	N	DAB
111	CC1	40 MINUTES	G219-1129	1/600	32 MINUTES	CELL MARQUE	1616010a	OPTIVIEW	Y	Y	DAB
112	BONE Epitope Rtrieval 2	30 minutes	FE11	1:100	30 minutes	DAKO	10109387	BOND Polymer Refine	none	none	DAB
114	CC1	32mins	G219-1129	1:100	32mins	Cell Marque	1505809 B	OPTIVIEW	N	N	DAB
115	envision flex high pH	30 mins	FE11	RTU	20 mins	Dako	10115752	envision flex	N	N	DAB
116	CC1	40 MIN	G219-1129	1/400	48 MIN	CELL MARQUE	1505809 F	OPTIVIEW DAB IHC	N	COPPER	DAB
123	FLEX HIGH pH	15 MIN	G219-1129	1/100	20 MIN	BDBIOSCIENCE	8329802	FLEX DAB	N	N	DAB
124	CC1	24 min	G219-1129	PrÃ©-diluÃ©	16 min	Cell Marque	1505808E	Optiview	non	non	Dab
125	HIGH pH	30 MIN	FE11	RTU	30 MIN	DAKO	10112557	ENVISION FLEX	N	N	DAB
126	Microwave Pressure Cooker, citrate buffer ph 6.0	34 minutes	FE11	1:75	30 MINUTES	DAKO	10111672	ULTRAVISION QUANTO DETECTION SYSTEM, HRP	YES	NO	DAB
138	HIER - EDTA	20	FE11	RTU	20	Dako	10112557	Polymer	Y	N	DAB
141	HIER	20	FE11	1:150	20	Dako	10106449	Polymer	Y	N	DAB
144	CC1	16 min	G219-1129	Pre-Dilute	16 mion	Cell Marque	1505808C	Optiview	No	Yes, copper	DAB
145	CC1	40	G219-1129	1/400	24	CELLMARQUE	1313003A	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min	FE11	RTU	20 min	Dako	10112557	EnVision Flex	Yes	No	DAB
175	HIER	32 min	G219-1129	predilute	16 min	Roche	1529502F	polymer	N	Y	DAB
181	HIER	20 minutes	FE11	1:150	20 minutes	DAKO	10106449	HRP-POLYMER	Y	N	DAB
186	HIER	20 MIN.	G219-1129	1:200	15 MIN.	CELL MARQUE	13334002F	LEICA BOND POLYMER	N	N	DAB
189	CC1	32	G219-1129	pre-dilute	16	Cell Marque	unknown	ultraView DAB	N	N	DAB
193	high pH	30 minutes	FE11	RTU	32	dako	unknown	flex +	no	no	DAB
202	ER2 Leica	20	25D12	1/50	15 min	leica	6039017	Refine Detection kit Leica	no	no	DAB
217	HIER	56	G2191129	predilute	32	Roche Ventana	1505808D	Optiview	N	Y	DAB
220	CC1	32min	G219-1129	prediluted	32min	Cell Marque	1505808E	OptiView	no	no	DAB
222	CC1	90min	G219-1129	RTU	12min	Roche	1505808E	Optiview DAB	Y	Copper	DAB

Table S4. Reported MSH6 staining protocols.

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time	Antibody Clone	Antibody Dilution	Antibody Incubation Time	Antibody Supplier	Antibody Lot #	Detection System	Amplification Y/N	Enhancement Y/N	Chromogen
101	CC1	32min	EP49	1:100	32 min @37	Epitomics	EN020910	Optiview	n	n	DAB
102	DAKO PT HIGH pH	10/20/10	EP49	1:100	30"RT	DAKO	10100346	DAKO FLEX	NO	CUS04	DAB+
103	CC1	1 HOUR	44	PRE	1 HOUR	VENTANA	F01005	OPTIVEIW	N	Y	DAB
104	HIER (heat induced epitope retrieval)	20 min	EP49	RTU	30 min	DAKO	10106765	polymere	no	no	DAB
106	microwave pressure cooker	30 min	SP93	1:15	45 minutes	Cell Marque	1526102B	MACH4	no	no	DAB
107	cc1	60 min	44	1:300	32 min	BD	5217835	Ultraview DAB	N	Y	DAB
109	HIER CC1 HIGH pH	64 MIN	44	RTU	20 MIN	VENTANA	F10103	OPTIVIEW	N	Y	DAB
110	Dako PT high ph	20 min @97C	EP49	1:200	30 MIN	DAKO	10106453	Dako Envision Flex	N	N	DAB
111	CC1	48 MINUTES	SP93	1/100	32 INUTES	CELL MARQUE	1512802B	OPTIVIEW	N	Y	DAB
112	BOND Epitope Retrieval 2	40 minutes	EP49	1:1500	30 minutes	Epitomics	EL120401	BOND Polymer Refine	none	none	DAB
114	CC1	64mins	EP49	1:200	32mins	EPITOMICS	CL090101	OPTIVIEW	N	N	DAB
115	envision flex high pH	30 mins	EP49	RTU	20 mins	Dako	10114392	envision flex	N	N	DAB
116	CC1	32 MIN	BC/44	1/400	48 MIN	BIOCARE MEDICALE	70814	OPTIVIEW DAB IHC	N	COPPER	DAB
123	FLEX HIGH	15 MIN	EPR3945	1/300	40 MIN	ABCAM	GR89185-14	FLEX DAB	Y	N	DAB
124	CC1	64 min	EP49	1/50	32 min	Dako	10109532	optiview	non	non	Dab
125	HIGH pH	30 MIN	EP49	RTU	I HOUR	DAKO	10116831	ENVISION FLEX	N	N	DAB
126	Microwave Pressure Cooker, citrate buffer ph 6.0	34 minutes	EP49	1:75	30 MINUTES	DAKO	10109532	ULTRAVISION QUANTO DETECTION SYSTEM, HRP	YES	NO	DAB
138	HIER - EDTA	20	EP49	RTU	20	Dako	10111640	Polymer	N	N	DAB
141	HIER	20	EP49	1:200	30	DAKO	10106453	Polymer	Y	N	DAB
144	CC1	32 min	EP49	1:100	32 min	Epitomics	20910	Optiview	No	Yes, copper	DAB
145	CC1	32	44	1/300	16	CELLMARQUE	1313501A	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min	EP49	RTU	20 min	Dako	10108534	EnVision Flex	No	No	DAB
175	HIER	64 min	44	1 in 100	24 min	Cell Marque	1112208C	polymer	Y	Y	DAB
181	HIER	20 MINUTES	EP49	1:200	30 MINUTES	DAKO	10106453	HRP-POLYMER	N	N	DAB
186	HIER	20 MIN.	BC/44	1:50	15 MIN.	BIOCARE MEDICAL	50815	LEICA BOND POLYMER	N	N	DAB
189	CC1	64	44	pre-dilute	16	Ventana	unknown	ultraView DAB	N	N	DAB
193	high pH	30 minutes	EP49	RTU	20 minutes	Dako	unknown	Flex +	No	No	DAB
202	ER2 Leica	40	pu29	1/25	15 min	abcam	gr262215	Refine Detection kit Leica	no	no	DAB
217	HIER	64	GTBP45	1:3000	60	Roche Ventana	FD2569	Optiview	N	Y	DAB
220	CC1	32min	EP49	1/50	28min	Dako	10100348	OptiView	N	N	DAB
222	CC1	90min	44	RTU	16min	Roche	F07076	Optiview DAB	Y	Copper	DAB

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	31	83.87	26	26/26 (100%)	1	1	1	1	1
102	31	90.32	28	28/28 (100%)	1	1	1	1	1
103	31	90.32	28	28/28 (100%)	1	1	1	1	1
104	31	96.77	30	30/30 (100%)	1	1	1	1	1
106	31	96.77	30	30/30 (100%)	1	1	1	1	1
107	31	100	31	31/31 (100%)	1	1	1	1	1
109	31	77.42	24	24/24 (100%)	1	1	1	1	1
110	31	90.32	28	28/28 (100%)	1	1	1	1	1
111	31	100	31	31/31 (100%)	1	1	1	1	1
112	31	96.77	30	30/30 (100%)	1	1	1	1	1
114	31	93.55	29	28/29 (97%)	0.92	1	1	0.94	0.93
115	31	87.1	27	27/27 (100%)	1	1	1	1	1
116	31	80.65	25	23/25 (92%)	0.91	0.93	0.91	0.93	0.84
123	31	96.77	30	29/30 (97%)	1	0.94	0.93	1	0.93
124	31	93.55	29	29/29 (100%)	1	1	1	1	1
125	31	96.77	30	30/30 (100%)	1	1	1	1	1
126	31	96.77	30	30/30 (100%)	1	1	1	1	1
138	31	93.55	29	29/29 (100%)	1	1	1	1	1
141	31	100	31	31/31 (100%)	1	1	1	1	1
144	31	96.77	30	30/30 (100%)	1	1	1	1	1
145	31	96.77	30	30/30 (100%)	1	1	1	1	1
149	31	93.55	29	28/29 (97%)	1	0.94	0.93	1	0.93
175	31	80.65	25	24/25 (96%)	0.92	1	1	0.92	0.92
181	31	93.55	29	29/29 (100%)	1	1	1	1	1
186	31	93.55	29	27/29 (93%)	1	0.88	0.86	1	0.86
189	31	87.1	27	26/27 (96%)	0.92	1	1	0.94	0.92
193	31	64.52	20	19/20 (95%)	0.91	1	1	0.9	0.9
202	31	83.87	26	24/26 (92%)	1	0.85	0.87	1	0.85
217	31	93.55	29	27/29 (93%)	1	0.87	0.88	1	0.86
220	31	93.55	29	29/29 (100%)	1	1	1	1	1
222	31	96.77	30	29/30 (97%)	1	0.94	0.93	1	0.93

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	32	84.38	27	26/27 (96%)	0.9	1	1	0.94	0.92
102	32	93.75	30	29/30 (97%)	0.92	1	1	0.95	0.93
103	32	37.5	12	11/12 (92%)	1	0	0.92	--	0
104	32	87.5	28	27/28 (96%)	0.92	1	1	0.94	0.93
106	32	96.88	31	31/31 (100%)	1	1	1	1	1
107	32	87.5	28	28/28 (100%)	1	1	1	1	1
109	32	81.25	26	25/26 (96%)	0.91	1	1	0.94	0.92
110	32	87.5	28	28/28 (100%)	1	1	1	1	1
111	32	93.75	30	29/30 (97%)	0.91	1	1	0.95	0.93
112	32	93.75	30	30/30 (100%)	1	1	1	1	1
114	32	87.5	28	27/28 (96%)	0.91	1	1	0.94	0.92
115	32	84.38	27	26/27 (96%)	0.92	1	1	0.94	0.92
116	32	90.63	29	28/29 (97%)	0.91	1	1	0.95	0.93
123	32	90.63	29	28/29 (97%)	0.92	1	1	0.94	0.93
124	32	90.63	29	28/29 (97%)	0.92	1	1	0.94	0.93
125	32	81.25	26	25/26 (96%)	0.91	1	1	0.94	0.92
126	32	93.75	30	29/30 (97%)	0.91	1	1	0.95	0.93
138	32	78.13	25	24/25 (96%)	0.91	1	1	0.93	0.92
141	32	93.75	30	30/30 (100%)	1	1	1	1	1
144	32	93.75	30	30/30 (100%)	1	1	1	1	1
145	32	93.75	30	29/30 (97%)	1	0.95	0.92	1	0.93
149	32	87.5	28	28/28 (100%)	1	1	1	1	1
175	32	78.13	25	25/25 (100%)	1	1	1	1	1
181	32	78.13	25	24/25 (96%)	0.91	1	1	0.93	0.92
186	32	90.63	29	28/29 (97%)	1	0.94	0.92	1	0.93
189	32	75	24	20/24 (83%)	0.9	0.79	0.75	0.92	0.67
193	32	50	16	16/16 (100%)	1	1	1	1	1
202	32	84.38	27	27/27 (100%)	1	1	1	1	1
217	32	87.5	28	27/28 (96%)	0.92	1	1	0.94	0.93
220	32	87.5	28	27/28 (96%)	0.9	1	1	0.95	0.92
222	32	21.88	7	6/7 (86%)	1	0	0.86	--	0

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	31	96.77	30	30/30 (100%)	1	1	1	1	1
102	31	96.77	30	30/30 (100%)	1	1	1	1	1
103	31	90.32	28	28/28 (100%)	1	1	1	1	1
104	31	93.55	29	29/29 (100%)	1	1	1	1	1
106	31	100	31	31/31 (100%)	1	1	1	1	1
107	31	67.74	21	21/21 (100%)	1	1	1	1	1
109	31	74.19	23	23/23 (100%)	1	1	1	1	1
110	31	100	31	31/31 (100%)	1	1	1	1	1
111	31	100	31	31/31 (100%)	1	1	1	1	1
112	31	96.77	30	30/30 (100%)	1	1	1	1	1
114	31	100	31	31/31 (100%)	1	1	1	1	1
115	31	87.1	27	27/27 (100%)	1	1	1	1	1
116	31	83.87	26	26/26 (100%)	1	1	1	1	1
123	31	100	31	31/31 (100%)	1	1	1	1	1
124	31	100	31	31/31 (100%)	1	1	1	1	1
125	31	100	31	31/31 (100%)	1	1	1	1	1
126	31	96.77	30	30/30 (100%)	1	1	1	1	1
138	31	100	31	31/31 (100%)	1	1	1	1	1
141	31	96.77	30	30/30 (100%)	1	1	1	1	1
144	31	100	31	31/31 (100%)	1	1	1	1	1
145	31	93.55	29	29/29 (100%)	1	1	1	1	1
149	31	100	31	31/31 (100%)	1	1	1	1	1
175	31	87.1	27	27/27 (100%)	1	1	1	1	1
181	31	93.55	29	29/29 (100%)	1	1	1	1	1
186	31	70.97	22	22/22 (100%)	1	1	1	1	1
189	31	67.74	21	21/21 (100%)	1	1	1	1	1
193	31	67.74	21	21/21 (100%)	1	1	1	1	1
202	31	90.32	28	28/28 (100%)	1	1	1	1	1
217	31	96.77	30	30/30 (100%)	1	1	1	1	1
220	31	96.77	30	30/30 (100%)	1	1	1	1	1
222	31	96.77	30	30/30 (100%)	1	1	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	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	29	96.55	28	27/28 (96%)	1	0.88	0.95	1	0.91
102	29	96.55	28	25/28 (89%)	1	0.63	0.87	1	0.7
103	29	89.66	26	24/26 (92%)	0.95	0.86	0.95	0.86	0.8
104	29	89.66	26	25/26 (96%)	1	0.83	0.95	1	0.88
106	29	93.1	27	27/27 (100%)	1	1	1	1	1
107	29	72.41	21	21/21 (100%)	1	1	1	1	1
109	29	93.1	27	26/27 (96%)	0.95	1	1	0.86	0.9
110	29	96.55	28	27/28 (96%)	1	0.88	0.95	1	0.91
111	29	100	29	27/29 (93%)	1	0.75	0.91	1	0.81
112	29	89.66	26	25/26 (96%)	0.95	1	1	0.83	0.88
114	29	100	29	27/29 (93%)	0.95	0.88	0.95	0.88	0.83
115	29	93.1	27	26/27 (96%)	0.95	1	1	0.86	0.9
116	29	89.66	26	24/26 (92%)	1	0.67	0.91	1	0.75
123	29	96.55	28	27/28 (96%)	0.95	1	1	0.88	0.91
124	29	96.55	28	25/28 (89%)	1	0.63	0.87	1	0.7
125	29	100	29	26/29 (90%)	1	0.63	0.88	1	0.71
126	29	89.66	26	25/26 (96%)	1	0.88	0.95	1	0.91
138	29	96.55	28	27/28 (96%)	0.95	1	1	0.88	0.91
141	29	82.76	24	22/24 (92%)	0.94	0.88	0.94	0.88	0.81
144	29	68.97	20	19/20 (95%)	0.95	1	1	0.5	0.64
145	29	93.1	27	26/27 (96%)	1	0.88	0.95	1	0.91
149	29	96.55	28	26/28 (93%)	0.95	0.88	0.95	0.88	0.83
175	29	89.66	26	25/26 (96%)	0.94	1	1	0.89	0.91
181	29	89.66	26	25/26 (96%)	0.95	1	1	0.88	0.91
186	29	96.55	28	28/28 (100%)	1	1	1	1	1
189	29	48.28	14	13/14 (93%)	0.92	1	1	0.5	0.63
193	29	89.66	26	25/26 (96%)	0.95	1	1	0.86	0.9
202	29	100	29	25/29 (86%)	1	0.5	0.84	1	0.59
217	29	96.55	28	26/28 (93%)	1	0.71	0.91	1	0.79
220	29	93.1	27	26/27 (96%)	0.95	1	1	0.88	0.91
222	29	96.55	28	27/28 (96%)	0.95	1	1	0.89	0.92