

Summary Report – Run 118 MMR (MLH1, PMS2, MSH2, MSH6)

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Overview

Participating laboratories were asked to stain a tissue microarray consisting of 30 carcinomas. Germline sequencing data was available to confirm MMR status. MMR immunohistochemistry is used for preliminary identification of Lynch Syndrome patients, acting as a screening test that is then followed by a definitive genetic test. It has also been recently shown to predict response to immune checkpoint inhibitors. Readout of MMR immunostaining consists of

- **Expressed (E)** = intact/positive staining of tumour cell nuclei, which is normal and indicative of a non-mutant corresponding gene
- **Absent (A)** = loss of expression/deficient in tumour cell nuclei, with positive staining of non-tumour cells, is indicative of an abnormal result
- **Failed (F)** = when there is no staining of tumour or normal cell nuclei, such that it is not possible to comment on MMR expression

As mentioned in previous reports, variable staining can be attributed to both quality of tissue material and the unusual biology of MMR protein expression. For example, 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). Weak, patchy MSH6 expression may also be associated with loss of MSH2 expression/germline MSH2 mutations. Note that this MSH6 staining is not “normal” as it is patchy and weaker than in control cells. In the past MMR immunostaining was interpreted as abnormal only if there was complete absence of staining. We now know that weak expression can be seen in association with MMR deficiency. As with Run 111 MMR, we refer you to guidelines on interpretation of MMR immunostaining developed by the British Association of Gynecological Pathologists, available to download online at <https://www.thebagp.org/resources/>. Although this document, which is copiously illustrated, focuses on endometrial carcinoma, the examples of challenges in MMR staining interpretation also are applicable to other tumor types.

Results

MLH1

Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Adequate	Weak; possible Ab dilution issue as an intensity gradient is observed from the top left corner of the array
102	Optimal	
106	Adequate	Multiple cores physically scrapped but still interpretable; cytoplasmic background
107	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	Slight cytoplasmic background
113	Optimal	Slight cytoplasmic background
114	Optimal	
120	Optimal	
123	Optimal	
125	Optimal	Slight cytoplasmic background
126	Optimal	Slightly weak
127	Sub-optimal	Heavy cytoplasmic background, requiring significant adjustment of interpretive threshold
136	Optimal	
138	--	Slide not available for assessment

Lab ID	IHC Status*	Comments
141	Optimal	
144	Optimal	Slightly weak
149	Optimal	
160	Optimal	
175	Adequate	Slightly weak staining; slight cytoplasmic background
181	Optimal	
186	Adequate	Slightly weak staining; cytoplasmic background
190	Optimal	
194	Optimal	
198	Optimal	Slightly weak
202	Optimal	Slight cytoplasmic background
207	Optimal	Slight cytoplasmic background
220	Adequate	Slightly weak staining; slight cytoplasmic background
222	Optimal	
230	Optimal	
231	Optimal	

*based on CPQA assessor consensus

MLH1 Garrattogram after CPQA assessment:

Lab/ Core	101	102	106	107	110	111	112	113	114	120	123	125	126	127	136	138	141	144	149	160	175	181	186	190	194	198	202	207	220	222	230	231	MMR Status				
1	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	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	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	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	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	U	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	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	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	E	E	E	MSH6		
9	E	E	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	E	E	E	E	E	PMS2		
10	E	E	E	E	U	E	E	E	E	E	E	E	E	U	U	U	U	U	U	U	U	U	U	E	E	E	U	U	U	U	U	U	U	U	Normal		
11	E	U	E	E	E	E	E	E	E	E	U	E	E	E	E	U	E	E	E	E	E	E	E	E	U	E	E	U	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	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	E	E	E	E	E	E	E	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	E	E	E	E	MSH2	
15	U	U	E	E	E	E	E	E	E	U	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E	U	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	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	F	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	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	E	E	E	E	E	Normal
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	E	E	E	E	E	Normal
21	E	E	U	E	E	E	E	E	E	E	F	E	E	F	E	E	E	E	E	E	E	E	E	E	F	F	E	E	E	E	F	E	E	E	E	MSH6	
22	U	U	U	F	A	A	F	A	A	U	U	U	U	A	A	A	A	A	A	A	A	A	A	A	U	U	A	A	A	A	A	A	A	A	A	A	MLH1
23	U	U	U	U	E	E	E	E	E	E	E	E	E	U	U	U	U	U	U	U	U	U	E	U	U	E	E	U	U	U	U	U	U	U	U	MSH2	
24	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	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	E	E	E	E	E	Normal
26	U	U	E	E	E	E	E	E	U	U	U	U	E	E	E	E	E	E	E	E	E	E	E	E	U	U	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	E	E	E	E	E	MSH6
28	U	U	U	E	U	U	U	U	U	U	U	U	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	E	E	E	E	E	Normal
29	E	E	E	E	E	E	E	E	E	E	F	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	F	E	E	PMS2	
30	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	F	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	F	A	A	MLH1	

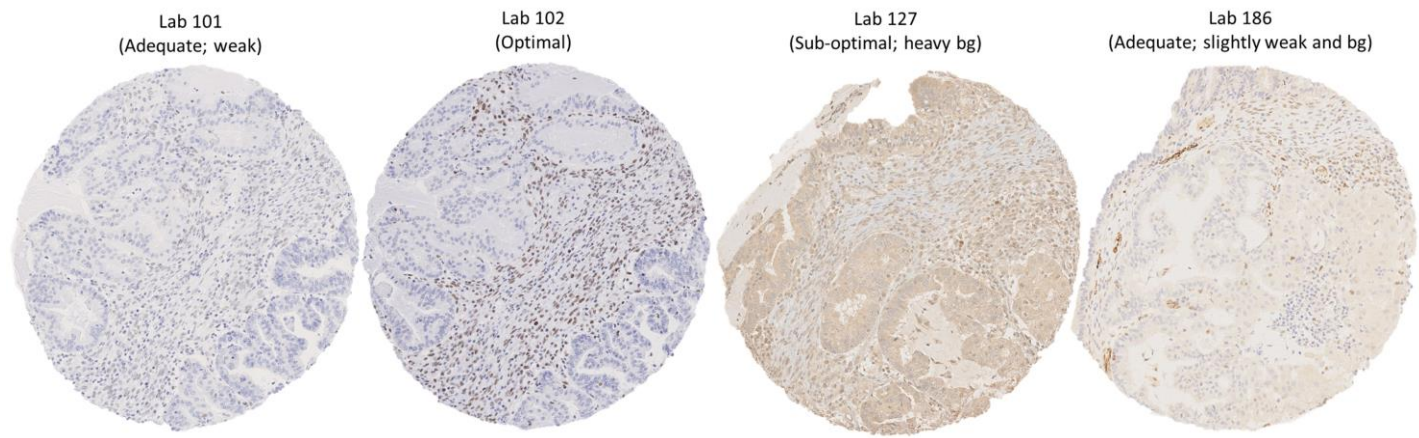


Figure 1. Representative images of the qualitative variability of MLH1 staining in a case with an MLH1 germline mutation (i.e. loss of expression). bg = background

PMS2

Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Optimal	Slightly weak
102	Optimal	
106	Adequate	Cytoplasmic background
107	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	Slide broken during return shipment but still interpretable; slightly weak
120	Optimal	
123	Optimal	
125	Optimal	Slightly weak
126	Sub-optimal	Very weak staining
127	Optimal	
136	Optimal	
138	--	Slide not available for assessment

Lab ID	IHC Status*	Comments
141	Optimal	Slightly weak
144	Optimal	
149	Optimal	Slight cytoplasmic background
160	Optimal	
175	Adequate	Slight cytoplasmic background and slightly weak staining
181	Optimal	
186	Optimal	Slight cytoplasmic background
190	Optimal	
194	Optimal	Slight cytoplasmic background
198	Adequate	Slight cytoplasmic background and slightly weak staining
202	Adequate	Slight cytoplasmic background and slightly weak staining
207	Optimal	Slightly weak
220	Optimal	Slightly weak
222	Optimal	
230	Optimal	
231	Optimal	

PMS2 Garrattogram after CPQA assessment:

Lab/ Core	101	102	106	107	110	111	112	113	114	120	123	125	126	127	136	138	141	144	149	160	175	181	186	190	194	198	202	207	220	222	230	231	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	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	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	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	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	U	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	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	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	E	MSH6
9	A	U	A	A	A	A	U	A	A	A	U	A	F	A	A	A	A	U	A	A	A	A	A	A	U	A	A	A	A	U	A	A	PMS2
10	E	E	E	E	E	E	E	E	E	E	E	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	Normal	
11	E	U	U	E	E	E	E	E	E	U	F	E	E	E	U	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	U	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	A	MLH1
13	E	E	E	E	E	E	E	E	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
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	E	MSH2
15	U	U	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	E	E	E	E	E	E	E	U	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	U	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	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	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	E	Normal
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	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	F	E	E	E	E	F	E	E	MSH6
22	U	U	U	A	A	A	F	U	A	U	A	U	F	A	A	A	A	A	A	A	A	A	A	F	U	A	A	A	A	F	A	A	MLH1
23	E	E	E	U	E	E	E	E	E	E	E	F	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	MSH2	
24	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	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	E	Normal
26	U	U	U	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	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	E	MSH6
28	U	U	U	E	U	U	U	U	U	U	U	U	E	E	E	E	E	E	E	E	E	E	E	U	E	E	U	E	E	E	E	Normal	
29	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	A	A	A	PMS2
30	A	A	A	A	A	A	A	A	A	A	A	F	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	MLH1

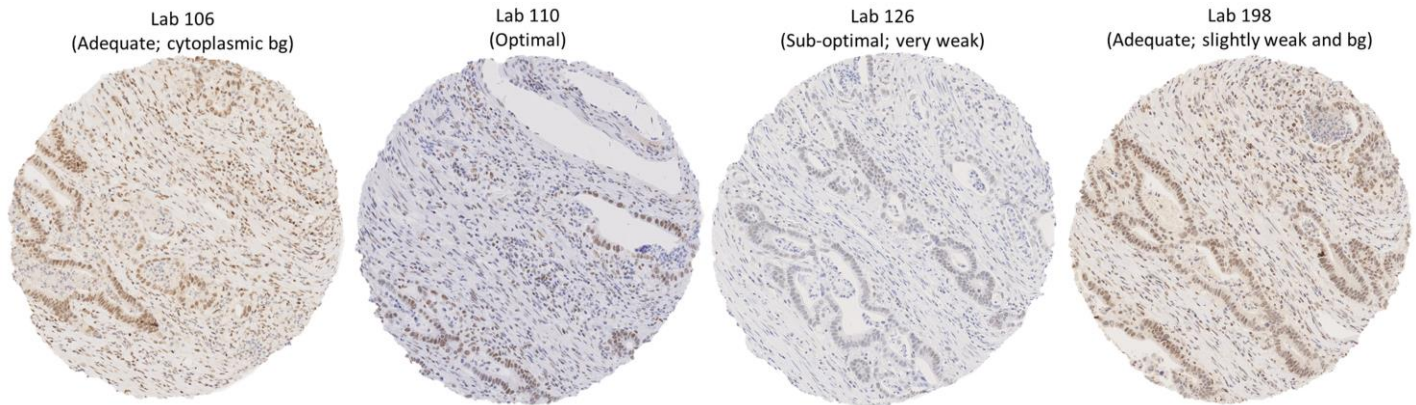


Figure 2. Representative images of the qualitative variability of PMS2 staining in a case with normal expression. bg = background

MSH2

Few tumour cells were noted in Core 18. Antigenicity was variable for Core 30 likely due to poor fixation. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	
106	Adequate	Cytoplasmic background
107	Optimal	
110	Optimal	
111	Adequate	Generally weak staining in most cores
112	Optimal	
113	Optimal	
114	Optimal	
120	Optimal	
123	Optimal	Slightly weak
125	Optimal	
126	Adequate	Weak staining
127	Optimal	Slight cytoplasmic background
136	Optimal	
138	--	Slide not available for assessment

Lab ID	IHC Status*	Comments
141	Adequate	Weak staining
144	Optimal	
149	Optimal	
160	Optimal	
175	Optimal	Slight cytoplasmic background in some cores
181	Optimal	
186	Optimal	Slightly weak
190	Optimal	Slightly weak
194	Optimal	
198	Optimal	
202	Optimal	Slight cytoplasmic background in some cores
207	Optimal	
220	Optimal	
222	Optimal	
230	Optimal	
231	Optimal	

MSH2 Garrattogram after CPQA assessment:

Lab/ Core	101	102	106	107	110	111	112	113	114	120	123	125	126	127	136	138	141	144	149	160	175	181	186	190	194	198	202	207	220	222	230	231	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	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	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	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	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	E	E	Normal		
6	E	E	E	E	E	F	E	E	E	E	F	E	E	E	E	E	F	E	E	E	E	E	E	F	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	E	E	E	Normal	
8	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	E	E	E	E	E	E	MSH6	
9	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	E	E	E	E	PMS2	
10	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	U	U	U	U	U	U	U	U	E	U	U	U	U	U	U	U	U	Normal		
11	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	U	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	E	E	E	MLH1	
13	A	A	A	A	A	A	A	A	A	A	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	
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	A	A	A	MSH2	
15	U	U	E	E	E	E	E	E	E	U	U	U	U	E	E	E	E	E	E	E	E	E	E	U	U	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	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	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	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	E	E	E	E	Normal
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	E	E	E	E	Normal
21	E	E	E	E	F	F	E	E	E	F	F	A	F	E	F	A	F	E	F	E	F	A	A	F	F	F	F	F	A	F	E	F	E	F	MSH6	
22	U	U	U	E	E	U	U	E	E	U	U	U	U	E	E	E	F	E	E	E	E	E	E	U	U	E	E	E	E	E	E	E	E	E	MLH1	
23	A	U	A	U	A	A	A	A	A	U	A	U	A	U	U	U	U	U	U	U	U	U	U	A	A	U	U	U	U	U	U	U	U	U	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	U	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	E	E	E	E	Normal
26	U	U	U	A	A	A	U	A	U	U	U	U	U	A	A	A	A	A	A	A	A	A	A	U	U	A	A	A	A	A	A	A	A	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	E	E	E	E	MSH6
28	U	U	U	E	U	U	U	U	U	U	U	U	U	E	E	E	E	E	E	E	E	E	E	U	U	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	E	E	E	E	PMS2
30	E	E	E	E	E	A	E	E	E	E	A	E	F	F	E	E	A	E	E	E	E	E	A	F	F	F	E	A	A	A	F	E	E	E	MLH1	

MSH6

Once again, antigenicity was variable for Core 30 likely due to poor fixation. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	Weak, patchy nuclear staining in some cores with loss of MSH2 is more prominent than other labs
106	Optimal	
107	Optimal	
110	Optimal	
111	Optimal	Slightly weak
112	Optimal	
113	Optimal	
114	Optimal	
120	Optimal	
123	Optimal	Weak, patchy nuclear staining in some cores with loss of MSH2 is more prominent than other labs
125	Optimal	
126	Adequate	Weak staining
127	Optimal	Weak, patchy nuclear staining in some cores with loss of MSH2 is more prominent than other labs
136	Optimal	
138	--	Slide not available for assessment

Lab ID	IHC Status*	Comments
141	Adequate	Weak staining
144	Optimal	
149	Optimal	Weak, patchy nuclear staining in some cores with loss of MSH2 is more prominent than other labs
160	Optimal	
175	Optimal	
181	Optimal	Slightly weak
186	Adequate	Cytoplasmic background; weak staining
190	Adequate	Weak staining
194	Optimal	
198	Optimal	
202	Optimal	
207	Optimal	
220	Optimal	
222	Optimal	
230	Optimal	Several readout errors corrected during CPQA assessment
231	Optimal	Weak, patchy nuclear staining in some cores with loss of MSH2 is more prominent than other labs; slight cytoplasmic background in some cores

MSH6 Garrattogram after CPQA assessment:

Lab/ Core	101	102	106	107	110	111	112	113	114	120	123	125	126	127	136	138	141	144	149	160	175	181	186	190	194	198	202	207	220	222	230	231	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	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	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	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	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	E	E	E	Normal		
6	A	A	A	A	A	A	A	A	A	A	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		
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	E	E	E	Normal		
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	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	E	E	E	PMS2		
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	E	E	Normal		
11	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	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	E	E	E	MLH1		
13	A	A	A	A	A	A	A	A	A	A	A	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	
14	A	A	A	A	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	MSH2	
15	U	U	U	A	F	U	A	U	U	U	U	U	A	A	A	A	A	A	A	A	A	A	A	A	U	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	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	E	E	E	E	Normal	
18	A	A	F	A	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	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	E	E	E	E	Normal	
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	E	E	E	E	Normal	
21	A	A	A	A	A	A	F	A	A	A	A	A	F	A	A	F	F	A	A	A	A	A	A	F	A	A	F	A	A	A	F	A	A	A	A	MSH6	
22	U	U	U	E	E	E	E	E	E	U	U	U	F	E	E	E	F	E	E	E	E	E	F	U	E	E	E	E	E	E	E	E	E	E	E	MLH1	
23	A	U	A	A	A	A	A	A	A	A	A	U	U	U	U	U	U	U	U	U	U	U	U	A	A	U	U	U	U	U	U	U	U	U	U	MSH2	
24	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	F	U	U	A	A	A	A	U	A	A	A	A	A	F	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	E	E	E	E	Normal	
26	U	U	U	A	A	A	A	U	U	U	U	U	F	A	A	A	F	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	MSH2	
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	A	A	A	A	A	A	MSH6
28	U	U	U	U	E	U	U	U	U	U	U	U	E	E	E	E	E	E	E	E	E	E	E	E	U	U	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	E	E	E	E	PMS2	
30	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	F	F	F	F	E	F	E	E	E	E	E	E	E	E	MLH1	

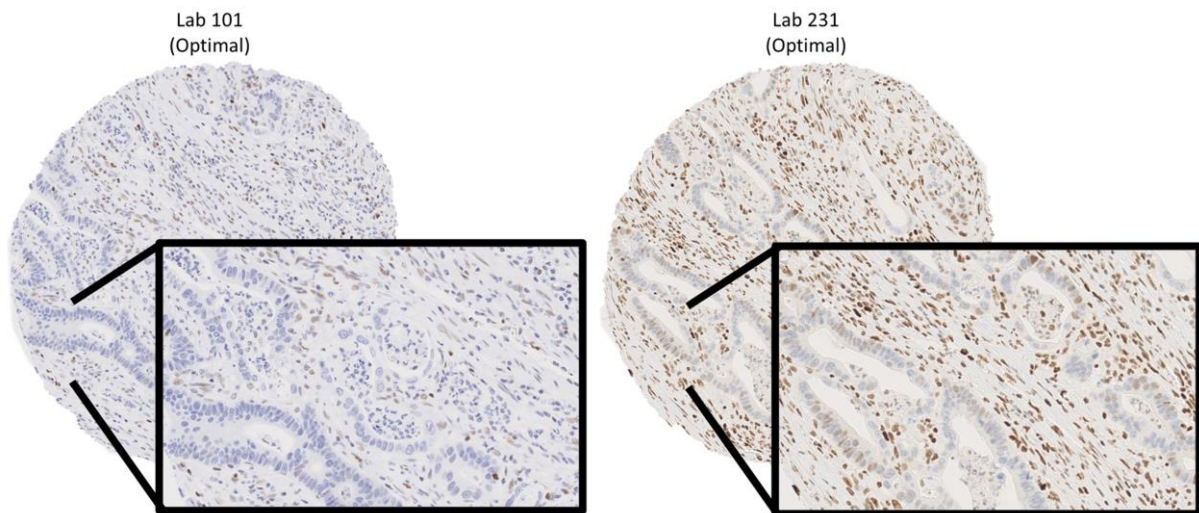


Figure 3. Representative images of weak, patchy MSH6 staining in tumour cell nuclei in a case with germline MSH2 mutation.

Supplementary Tables 1-4 summarize the reported staining protocols for MLH1, PMS2, MSH2, and MSH6, respectively, which can be referred to during validation or optimization of a staining protocol. Supplementary Tables 5-8 provide descriptive statistics for MLH1, PMS2, MSH2, and MSH6, respectively. Supplementary Table 9 provides the definitions of IHC Status and recommended participant action. Images in this document were updated using scanned images acquired using a NanoZoomer SQ that was kindly loaned to the CPQA-AQCP by Hamamatsu and Quorum Technologies as in-kind support. Your regular participation in CPQA-AQCP is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association canadienne des pathologistes.

This report has been updated with scanned images that were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.

Table S1. Reported MLH1 staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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)
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	ES05	RTU	DAKO	11123422	20 MIN	DAKO Envision FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retrieval solution 9.0	20	ESO5	1/80	Agilent	10153053	30" RT	Dako Envision Flex	Y	yes CuSO4
106	Dako Omnis	LDT	High pH buffer 97 deg	60	ESO5	RTU	Agilent/Dako	11155255	60	Dako Flex Plus	Yes	No
107	Dako Omnis	LDT	Dako FLEX TRS High pH	30	ESO5	RTU	Dako	11123422	30	Dako FLEX	N	N
110	DAKO Autostainer Link 48	LDT	DAKO PT High ph 9.0@97 C	20 min	ESO5	1:25	DAKO	11097054	20 min	Dako Envision Flex	Y	N
111	ULTRA BENCHMARK	COMMERCIAL	HIER	48	G168-15	1/50	BIOCARE	91919	32	OPTIVIEW	Y	Y
112	BOND III	LDT	BOND Epitope Retrieval 2 pH 9.0	25 minutes	ES05	1:100	Leica (Novocastra)	6074018	30 minutes	BOND Polymer Refine Detection	No	No
113	Dako Omnis	LDT	High pH	45 min	Es05	1:2 bkgd reducing diluent	Dako	11097054	32.5 min	Dako Envision Flex HRP	N	N
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	ES05	1:25	Dako	10151727	15	Envision FLEX DAKO Omnis	Y	N
120	Autostainer Link48	Commercial assay	HIER Waterbath	20	ES05	RTU	Dako	10154082	30	Dako Envision Flex	Y	N
123	Dako AutoStainer	LDT	FLEX TRS High	20	ES05	predilute	Dako	11123422	20	Flex+20 Mouse	Y	N
125	Dako Omnis	LDT	Hier	30	ES05	RTU	Dako	10151487	30	EnVision Flex	Y	N
126	Biocare intelliPATH	LDT	Pressure Cooker pH 6.0	8	ES05	1:50	Dako	10153053	30	Mach 4	N	N
127	Ventana Benchmark Ultra	Commercial Assay	HEIR	92 Min.	ES05	Predilute	Agilent	10151487	120 Min.	Ultraview DAB	Y	Y
136	DAKO AS480	LDT	DAKO PT HIGH PH	20	ES05	RTU	DAKO	11155255	15	DAKO ENVISION FLEX +	Y	N
138	Dako Autostainer Link 48	LDT	EDTA HIER	20	ES05	RTU	Dako	11123422	20	Polymer/Dako	y	N
141	Autostainer Link48	LDT	HIER	15	ES05	1:25	Dako	11097054	30	Polymer	Y	N
144	Dako Omnis	LDT	HIER	30	ESO5	RTU	Dako	10151487	40	Flex	Y	N
149	Dako OMNIS	LDT	high pH OMNIS	30 min at 97 C	ES05	RTU	Dako Agilent	11100169	30	EnVision Flex OMNIS	Yes	No
160	VENTANA	LDT	CC1	64MN	6168-15	1/100	BIOCARE	91919	1h	optiview	N	Y
175	Benchmark ULTRA	LDT	HIER	64	M1	Pre-dilute	Roche	F25054	16	OptiDAB	N	Y
181	Dako Autostainer link48	LDT	HIER pH9	20 min	ES05	1:25	DAKO	11097054	20 min	EnVision Flex	Y	N
186	LEICA BOND III	LDT	HIER	20	G168-728	1:50	CELL MARQUE	39750	15	BOND POLYMER REFINE DETECTION	N	N
190	Ventana Benchmark Ultra	LDT	HIER	40	ES05	1:50	Dako	10127457	32	DAB	N	N
194	ULTRA	LDT	HERI - CC1	64	M1	RTU	ROCHE	G03098	16	OPTIVIEW	N	Y
198	Omnis	LDT	HIER	30	ES05	RTU	Dako-Agilent	11155255	35	Dako Envision Flex	Y	N
202	Leica Bond III	LDT	HIER PH9.0	20	ESO5	50	NCL-Leica	6067621	15	Bond refine detection kit	N	N
207	Dako-Omnis	LDT	on line high pH	30	ES05	ready to use	Dako	11100169	25	Envision Flex DAB	Y	N
220	Ventana BenchMark Ultra	LDT	HIER	72	ESO5	1/25	DAKO	10153053	60	VENTANA OPTIVIEW	N	Y
222	Benchmark Ultra	commercial assay	Ultra CC1	40	M1	RTU	Roche	F29081	4	Optiview DAB	Y	Y
230	Benchmark Ultra	LDT	HIER	64	ES05	predilute	DAKO	11100169	40	OPTIVIEW	Y	N
231	Ventana BenchMark Ultra	LDT	HIER	64 mins	M1	RTU	VENTANA/ROCHE	F20834	40	OPTIVIEW (VENTANA)	N	Y

Table S2. Reported PMS2 staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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)
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	EP51	RTU	DAKO	11118110	20 MIN	DAKO Envision FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retrieval solution 9.0	20	EP51	1/20	Agilent	11097056	30" RT	Dako Envision Flex	N	yes CuSO4
106	Dako Omnis	LDT	High pH buffer 97 deg	60	EP51	RTU	Agilent/Dako	11123428	20	Dako Flex Plus	Yes	No
107	Dako Omnis	LDT	Dako FLEX TRS High pH	30	EP51	RTU	Dako	11123428	30	Dako FLEX	N	N
110	DAKO Autostainer Link 48	LDT	DAKO PT High ph 9.0@97 C	20 min	EP51	1:50	DAKO	11086006	30 min	Dako Envision Flex	N	N
111	ULTRA BENCHMARK	COMMERCIAL	HIER	48	EP51	1/100	DAKO	11086006	32	OPTIVIEW	Y	Y
112	BOND III	LDT	BOND Epitope Retrieval 2 pH 9.0	30 minutes	EP51	1:75 using powervision super blocking diluent	DAKO	11129038	30 minutes	BOND polymer refine	No	No
113	Ventana Ultra	LDT	CC1	64 min	EP51	1:60	Dako	11097056	32 min	Optiview	Y 8 min (Optiview Amp Kit)	N
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	EP51	RTU	Dako	10151727	30	Envision FLEX DAKO Omnis	Y	N
120	Autostainer Link48	Commercial assay	HIER Waterbath	20	EP51	RTU	Dako	10150190	30	Dako Envision Flex	Y	N
123	Dako Autostainer48	LDT	FLEX TRS High	20	A16-4	1/50	BD Pharinogen	64355	30	Flex+30 Mouse	Y	N
125	Dako	LDT	Hier	30	EP51	RTU	Dako	11118110	30	EnVision Flex	Y	N
126	Biocare intelliPATH	LDT	Pressure Cooker pH 6.0	8	EP51	1:50	Dako	10149140	30	Mach 4	N	N
127	Ventana Benchmark Ultra	Commercial Assay	HEIR	92 Min.	A16-4	Predilute	Ventana	G03092	32 Min.	Optiview DAB	Y	Y
136	DAKO AS480	LDT	DAKO PT HIGH PH	20	EP51	RTU	DAKO	11092475	30	DAKO ENVISION FLEX +	N	N
138	Dako Autostainer Link 48	LDT	EDTA HIER	20	EP51	RTU	Dako	11123428	20	Polymer/Dako	y	N
141	Autostainer Link48	LDT	HIER	15	EP51	1:50	Dako	11086006	30	Polymer	Y	N
144	Dako Omnis	Commercial Assay	HIER	30	EP51	RTU	Dako	10151727	40	Flex	Y	N
149	Dako OMNIS	LDT	high pH OMNIS	30 min at 97 C	EP51	RTU	Dako Agilent	11118110	30	EnVision Flex OMNIS	Yes	No
160	vENTANA	LDT	CC1	48mn	EP51	1/100	DAKO	11086006	32MN	optiview	Y	Y
175	Benchmark ULTRA	LDT	HIER	92	A16-4	Pre-dilute	Roche	F28271	32 min	Opti-DAB	Y	Y
181	Dako autostainer link48	LDT	HIER pH9	20 min	EP51	1:50	DAKO	11086006	30 min	EnVision Flex	N	N
186	LEICA BOND III	LDT	HIER	20	ERP3947	1:4	CELL MARQUE	37424	15	BOND POLYMER REFINE DETECTION	N	N
190	Ventana Benchmark Ultra	LDT	HIER	32	A16-4	Predilute	Roche	F28271	32	DAB	Y	N
194	ULTRA	LDT	HIER - CC1	92	A16-4	RTU	ROCHE	F28271	32	OPTIVIEW	N	Y
198	Dako-Omnis	LDT	HIER	30	EP51	RTU	Dako-Agilent	10151727	35	Dako Envision Flex	Y	N
202	Leica Bond III	LDT	HIER PH9.0	30	A16-4	25	BDPharm	64355	15	Bond polymer refine detecton kit	N	N
207	Dako-Omnis	LTD	on line high pH	30	EP51	ready to use	Dako	11118110	20	Envision Flex DAB	N	N
220	Ventana BenchMark Ultra	LDT	HIER	64	EP51	1/40	DAKO	10146840	68	VENTANA OPTIVIEW	N	Y
222	Benchmark Ultra	commercial assay	Ultra CC1	92	A16-4	RTU	Roche	F12418M	32	Optiview DAB	Y	Y
230	Benchmark Ultra	LDT	HIER	64	A16-4	predilute	Roche Diagnostics	F25056	32	OPTIVIEW	Y	N
231	Ventana BenchMark Ultra	LDT	HIER	72 MINS	EP 51	1/50	DAKO/AGILE NT	11086006	12 MINS	OPTIVIEW (VENTANA)	Y	Y

Table S3. Reported MSH2 staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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)
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	FE11	RTU	DAKO	10133138	30 MIN	DAKO Envision FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retrieval solution 9.0	20	FE11	1/40	Agilent	11098480	30" RT	Dako Envision Flex	Y	yes CuSO4
106	Dako Omnis	LDT	High pH buffer 97 deg	60	FE11	RTU	Agilent/Dako	11123426	30	Dako Flex Plus	Yes	No
107	Dako Omnis	LDT	Dako FLEX TRS High pH	30	FE11	RTU	Dako	10154166	25	Dako FLEX	N	N
110	DAKO Autostainer Link 48	LDT	DAKO PT High ph 9.0@97 C	20 min	FE11	1:200	DAKO	11085711	20 min	Dako Envision Flex	Y	N
111	ULTRA BENCHMARK	COMMERCIAL	HIER	40	G219-1129	1/600	CELL MARQUE	77805	32	OPTIVIEW	Y	Y
112	BOND III	LDT	BOND Epitope Retrieval Solution 2 pH 9.0	30 minutes	FE11	1:150	DAKO	11129034	30 minutes	BOND Polymer Refine Detection	No	No
113	Dako Omnis	LDT	High pH	45 min	G219-1129	1:400 bkgd reducing diluent	BD Pharmingen	9018901	25 min	Dako Envision Flex HRP	N	N
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	FE11	RTU	Dako	10148024	30	Envision FLEX DAKO Omnis	Y	N
120	Autostainer Link48	Commercial assay	HIER Waterbath	20	FE11	RTU	Dako	11092473	20	Dako Envision Flex	Y	N
123	Dako AutoStainer48	LDT	FLEX TRS High	20	G219-1129	1/100	BD Pharmingen	9018901	20	Flex 30	N	N
125	Dako Omnis	LDT	Hier	30	FE11	RTU	Dako	10148024	30	EnVision Flex	Y	N
126	Biocare intelliPATH	LDT	Pressure Cooker pH 6.0	8	FE11	1:200	Dako	10151760	30	Mach 4	N	N
127	Ventana Benchmark Ultra	Commercial Assay	HEIR	36 Min.	G219-1129	Predilute	Ventana	F29067	40 Min.	Ultraview DAB	Y	Y
136	DAKO AS480	LDT	DAKO PT HIGH PH	20	FE11	RTU	DAKO	11092473	30	DAKO ENVISION FLEX +	N	N
138	Dako Autostainer Link 48	LDT	EDTA HIER	20	FE11	RTU	Dako	11123426	20	Polymer/Dako	y	N
141	Autostainer Link48	LDT	HIER	15	FE11	1:200	Dako	11085711	20	Polymer	Y	N
144	Dako Omnis	LDT	HIER	30	FE11	RTU	Dako	10151725	35	Flex	Y	N
149	Dako OMNIS	LDT	high pH OMNIS	30 min at 97 C	FE11	RTU	Dako Agilent	11092473	30	EnVision Flex OMNIS	Yes	No
160	VENTANA	LDT	CC1	40MN	G219-1129	RTU	VENTANA	G03098	12MN	optiview	N	Y
175	Benchmark ULTRA	LDT	HIER	32 min	9219-1129	Pre-dilute	Roche	F29067	16	Opti-DAB	N	Y
181	Dako Autostainer link48	LDT	HIER pH9	20 min	FE11	1:200	DAKO	11085711	20 min	EnVision Flex	Y	N
186	LEICA BOND III	LDT	HIER	20	G219-1129	1:200	CELL MARQUE	25855	15	BOND POLYMER REFINE DETECTION	N	N
190	Ventana Benchmark Ultra	LDT	HIER	32	G219-1129	1:100	Cell Marque	5920	32	DAB	N	N
194	ULTRA	LDT	HIER - CC1	40	G219-1129	RTU	ROCHE	G03098	16	OPTIVIEW	N	Y
198	Dako-Omnis	LDT	HIER	30	FE11	RTU	Dako-Agilent	10148024	30	Dako-Envision Flex	Y	N
202	Leica Bond III	LDT	HIER PH9.0	20	G219-1129	100	Cellmarque	26488	15	Bond polymer refine detector kit	N	N
207	Dako-Omnis	LDT	on line high pH	30	FE11	ready to use	Dako	11088756	30	Envision Flex DAB	N	N
220	Ventana BenchMark Ultra	commercial assay	HIER	40	G219-1129	PRE DILUTE	VENTANA	F29067	12	VENTANA OPTIVIEW	N	Y
222	Benchmark Ultra	commercial assay	Ultra CC1	64	G219-1129	RTU	Cell Marque	F29067	24	Optiview DAB	N	Y
230	Benchmark Ultra	LDT	HIER	64	G219-1129	predilute	Roche Diagnostics	G03098	32	OPTIVIEW	N	N
231	Ventana BenchMark Ultra	LDT	HIER	40 MINS	G219-1129	RTU	ROCHE/VENTANA	F22808	32 mins	OPTIVIEW (VENTANA)	N	Y

Table S4. Reported MSH6 staining protocols.

Lab ID	Platform/instrument	LDT or commercial assay	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)
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	EP49	RTU	DAKO	11102760	20 MIN	DAKO Envision FLEX	N	N
102	Dako Autostainer 48 Link	LDT	Dako retrieval solution 9.0	20	EP49	1/100	Agilent	11086003	30" RT	Dako Envision Flex	N	yes CuSO4
106	Dako Omnis	LDT	High pH buffer 97 deg	60	EP49	RTU	Agilent/Dako	11146817	30	Dako Flex Plus	Yes	No
107	Dako Omnis	LDT	Dako FLEX TRS High pH	30	EP49	RTU	Dako	11127663	20	Dako FLEX	N	N
110	DAKO Autostainer Link 48	LDT	DAKO PT High ph 9.0@97 C	20 min	EP49	1:300	DAKO	11086003	30 min	Dako Envision Flex	N	N
111	ULTRA BENCHMARK	COMMERCIAL	HIER	32	SP93	1/100	CELL MARQUE	25792	32	OPTIVIEW	N	Y
112	BOND III	LDT	BOND Epitope Retrieval Solution 2 pH 9.0	40 minutes	EP49	1:1500	Epitomics/Cel I Marque	2E+08	30 minutes	BOND Polymer Refine Detection	No	No
113	Dako Omnis	LDT	High pH	45 min	EP49	RTU	Dako	11102760	25 min	Dako Envision Flex HRP	N	N
114	Dako Omnis	LDT	Envision Flex TRS, High pH	30	EP49	RTU	Dako	10147139	20	Envision FLEX DAKO Omnis	N	N
120	Autostainer Link48	Commercial assay	HIER Waterbath	20	EP49	RTU	Dako	10151006	30	Dako Envision Flex	N	N
123	Dako AutoStainer	LDT	FLEX TRS High	20	EPR3945	1/1200	abcam	GR32598 1	40	Flex +20 Rabbit	Y	N
125	Dako Omnis	LDT	Hier	30	EP49	RTU	Dako	11102760	10	EnVision Flex	Y	N
126	Biocare IntelliPATH	LDT	Pressure Cooker pH 6.0	8	EP49	1:75	Dako	11086003	30	Mach 4	N	N
127	Ventana Benchmark Ultra	Commercial Assay	HEIR	64 Min.	EP49	Predilute	Agilent	1.02E+08	32 Min.	Ultraview DAB	Y	Y
136	DAKO AS480	LDT	DAKO PT HIGH PH	20	EP49	RTU	DAKO	11088756	10	DAKO ENVISION FLEX +	N	N
138	Dako Autostainer Link 48	LDT	EDTA HIER	20	EP49	RTU	Dako	11102760	20	Polymer/Dako	N	N
141	Autostainer Link48	LDT	HIER	15	EP49	1:300	Dako	11086003	30	Polymer	Y	N
144	Dako Omnis	LDT	HIER	30	EP49	RTU	Dako	11127663	40	Flex	N	N
149	Dako OMNIS	LDT	high pH OMNIS	30 min at 97 C	EP49	RTU	Dako Agilent	1102760	30	EnVision Flex OMNIS	Yes	No
160	VENTANA	LDT	CC1	56MN	EPR3945	1/100	ABCAM	GR32598 10	32MN	optiview	N	Y
175	Benchmark ULTRA	LDT	HIER	64	SP93	Pre-dilute	Roche	F27678	12	Opti-DAB	N	Y
181	Dako Autostainer link48	LDT	HIER Ph9	20 min	EP49	1:300	DAKO	11086003	30 min	EnVision Flex	N	N
186	LEICA BOND III	LDT	HIER	20	44	1:50	BIOCARE MEDICAL	72519-3	15	BOND POLYMER REFINE DETECTION	N	N
190	Ventana Benchmark Ultra	LDT	HIER	56	EP49	1:750	Abcam	GR32598 10	32	DAB	N	N
194	ULTRA	LDT	HIER - CC1	56	SP93	RTU	ROCHE	F27678	8	OPTIVIEW	N	Y
198	Dako-Omnis	LDT	HIER	30	EP49	RTU	Dako-Agilent	10147139	30	Dako-Envision Flex	N	N
202	Leica Bond III	LDT	HIER PH 6.0	20	EP49	RTU	Dako/Agilent	11102760	15	Bond polymer refine detecton kit	N	N
207	Dako-Omnis	LDT	on line high pH	30	EP49	ready to use	DAko	11088756	20	Envision Flex DAB	N	N
220	Ventana BenchMark Ultra	LDT	HIER	32	EP49	1/50	DAKO	10149802	28	VENTANA OPTIVIEW	N	Y
222	Benchmark Ultra	commercial assay	Ultra CC1	64	sP63	RTU	Roche	F27678	12	Optiview DAB	N	Y
230	Benchmark Ultra	LDT	HIER	64	EP49	predilute	DAKO	10152980	32	OPTIVIEW	N	N
231	Ventana BenchMark Ultra	LDT	HIER	64 mins	SP93	RTU	ROCHE/VEN TANA	F07963	24 mins	OPTIVIEW (VENTANA)	N	Y

Table S5. Descriptive statistics for MLH1.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	30	83.33	25	25/25 (100%)	1	1	1
102	30	80	24	24/24 (100%)	1	1	1
106	30	80	24	24/24 (100%)	1	1	1
107	30	93.33	28	28/28 (100%)	1	1	1
110	30	93.33	28	28/28 (100%)	1	1	1
111	30	96.67	29	29/29 (100%)	1	1	1
112	30	93.33	28	28/28 (100%)	1	1	1
113	30	96.67	29	29/29 (100%)	1	1	1
114	30	93.33	28	28/28 (100%)	1	1	1
120	30	86.67	26	26/26 (100%)	1	1	1
123	30	76.67	23	23/23 (100%)	1	1	1
125	30	86.67	26	26/26 (100%)	1	1	1
126	30	83.33	25	25/25 (100%)	1	1	1
127	30	86.67	26	26/26 (100%)	1	1	1
136	30	93.33	28	28/28 (100%)	1	1	1
138	30	86.67	26	26/26 (100%)	1	1	1
141	30	93.33	28	28/28 (100%)	1	1	1
144	30	93.33	28	28/28 (100%)	1	1	1
149	30	90	27	27/27 (100%)	1	1	1
160	30	93.33	28	28/28 (100%)	1	1	1
175	30	96.67	29	29/29 (100%)	1	1	1
181	30	93.33	28	28/28 (100%)	1	1	1
186	30	93.33	28	28/28 (100%)	1	1	1
190	30	86.67	26	26/26 (100%)	1	1	1
194	30	70	21	21/21 (100%)	1	1	1
198	30	93.33	28	28/28 (100%)	1	1	1
202	30	90	27	27/27 (100%)	1	1	1
207	30	90	27	27/27 (100%)	1	1	1
220	30	93.33	28	28/28 (100%)	1	1	1
222	30	83.33	25	25/25 (100%)	1	1	1
230	30	90	27	27/27 (100%)	1	1	1
231	30	93.33	28	28/28 (100%)	1	1	1

Table S6. Descriptive statistics for PMS2.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	30	86.67	26	26/26 (100%)	1	1	1
102	30	80	24	24/24 (100%)	1	1	1
106	30	86.67	26	26/26 (100%)	1	1	1
107	30	96.67	29	29/29 (100%)	1	1	1
110	30	96.67	29	29/29 (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	93.33	28	28/28 (100%)	1	1	1
114	30	96.67	29	29/29 (100%)	1	1	1
120	30	83.33	25	25/25 (100%)	1	1	1
123	30	86.67	26	26/26 (100%)	1	1	1
125	30	86.67	26	26/26 (100%)	1	1	1
126	30	80	24	24/24 (100%)	1	1	1
127	30	93.33	28	28/28 (100%)	1	1	1
136	30	93.33	28	28/28 (100%)	1	1	1
138	30	86.67	26	26/26 (100%)	1	1	1
141	30	93.33	28	28/28 (100%)	1	1	1
144	30	90	27	27/27 (100%)	1	1	1
149	30	86.67	26	26/26 (100%)	1	1	1
160	30	93.33	28	28/28 (100%)	1	1	1
175	30	96.67	29	29/29 (100%)	1	1	1
181	30	93.33	28	28/28 (100%)	1	1	1
186	30	93.33	28	28/28 (100%)	1	1	1
190	30	86.67	26	26/26 (100%)	1	1	1
194	30	73.33	22	22/22 (100%)	1	1	1
198	30	93.33	28	28/28 (100%)	1	1	1
202	30	93.33	28	28/28 (100%)	1	1	1
207	30	96.67	29	29/29 (100%)	1	1	1
220	30	93.33	28	28/28 (100%)	1	1	1
222	30	83.33	25	25/25 (100%)	1	1	1
230	30	90	27	27/27 (100%)	1	1	1
231	30	93.33	28	28/28 (100%)	1	1	1

Table S7. Descriptive statistics for MSH2. Cores 18 and 30 were excluded from analysis for reasons noted above.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	28	85.71	24	24/24 (100%)	1	1	1
102	28	82.14	23	23/23 (100%)	1	1	1
106	28	85.71	24	24/24 (100%)	1	1	1
107	28	96.43	27	27/27 (100%)	1	1	1
110	28	96.43	27	27/27 (100%)	1	1	1
111	28	89.29	25	25/25 (100%)	1	1	1
112	28	89.29	25	25/25 (100%)	1	1	1
113	28	96.43	27	27/27 (100%)	1	1	1
114	28	92.86	26	26/26 (100%)	1	1	1
120	28	82.14	23	23/23 (100%)	1	1	1
123	28	78.57	22	22/22 (100%)	1	1	1
125	28	82.14	23	23/23 (100%)	1	1	1
126	28	85.71	24	24/24 (100%)	1	1	1
127	28	96.43	27	27/27 (100%)	1	1	1
136	28	92.86	26	26/26 (100%)	1	1	1
138	28	85.71	24	24/24 (100%)	1	1	1
141	28	85.71	24	24/24 (100%)	1	1	1
144	28	92.86	26	26/26 (100%)	1	1	1
149	28	89.29	25	25/25 (100%)	1	1	1
160	28	89.29	25	25/25 (100%)	1	1	1
175	28	92.86	26	26/26 (100%)	1	1	1
181	28	92.86	26	26/26 (100%)	1	1	1
186	28	92.86	26	26/26 (100%)	1	1	1
190	28	78.57	22	22/22 (100%)	1	1	1
194	28	75	21	21/21 (100%)	1	1	1
198	28	92.86	26	26/26 (100%)	1	1	1
202	28	92.86	26	26/26 (100%)	1	1	1
207	28	92.86	26	26/26 (100%)	1	1	1
220	28	92.86	26	26/26 (100%)	1	1	1
222	28	92.86	26	26/26 (100%)	1	1	1
230	28	92.86	26	26/26 (100%)	1	1	1
231	28	92.86	26	26/26 (100%)	1	1	1

Table S8. Descriptive statistics for MSH6. Core 30 was excluded from analysis for reasons noted above.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	29	86.21	25	25/25 (100%)	1	1	1
102	29	82.76	24	24/24 (100%)	1	1	1
106	29	79.31	23	23/23 (100%)	1	1	1
107	29	96.55	28	28/28 (100%)	1	1	1
110	29	96.55	28	28/28 (100%)	1	1	1
111	29	93.1	27	27/27 (100%)	1	1	1
112	29	93.1	27	27/27 (100%)	1	1	1
113	29	89.66	26	26/26 (100%)	1	1	1
114	29	89.66	26	26/26 (100%)	1	1	1
120	29	86.21	25	25/25 (100%)	1	1	1
123	29	82.76	24	24/24 (100%)	1	1	1
125	29	82.76	24	24/24 (100%)	1	1	1
126	29	82.76	24	24/24 (100%)	1	1	1
127	29	89.66	26	26/26 (100%)	1	1	1
136	29	93.1	27	27/27 (100%)	1	1	1
138	29	86.21	25	25/25 (100%)	1	1	1
141	29	82.76	24	24/24 (100%)	1	1	1
144	29	86.21	25	25/25 (100%)	1	1	1
149	29	86.21	25	25/25 (100%)	1	1	1
160	29	89.66	26	26/26 (100%)	1	1	1
175	29	93.1	27	27/27 (100%)	1	1	1
181	29	93.1	27	27/27 (100%)	1	1	1
186	29	89.66	26	26/26 (100%)	1	1	1
190	29	82.76	24	24/24 (100%)	1	1	1
194	29	89.66	26	26/26 (100%)	1	1	1
198	29	93.1	27	27/27 (100%)	1	1	1
202	29	89.66	26	26/26 (100%)	1	1	1
207	29	93.1	27	27/27 (100%)	1	1	1
220	29	93.1	27	27/27 (100%)	1	1	1
222	29	89.66	26	26/26 (100%)	1	1	1
230	29	89.66	26	26/26 (100%)	1	1	1
231	29	93.1	27	27/27 (100%)	1	1	1

Table S9. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining).	PASS
Adequate	All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation.	PASS
Sub-optimal	None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation.	PASS, Conditionally¹
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	FAIL²
Unsatisfactory	Technical issue (e.g. unsuitable antibody selection, etc.)	N/A

1 – A one-time sub-optimal performance qualifies for a “Pass” result. Two successive “sub-optimal” results will be designated as a “Fail”.

1,2 – Please contact the CPQA-AQCP for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory’s accreditation provider.