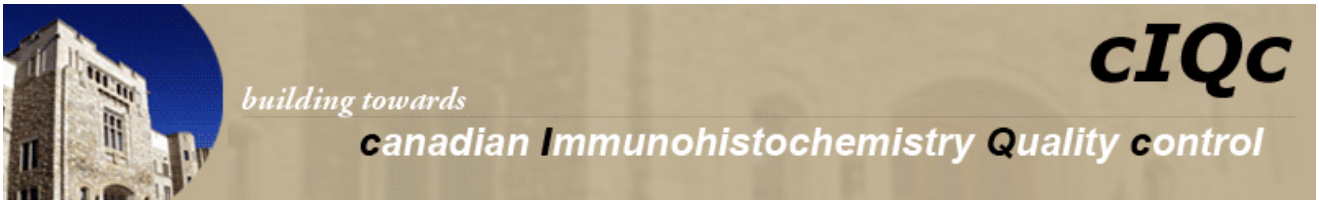

Assessors' report for cIQc Run 38: MMR immunostaining (May 2014)

Assessors: B Gilks, J Garratt and E Torlakovic

Assessment performed on Friday, August 29, 2014, at UKNEQAS Headquarters, London, UK

Background

MMR immunostaining is rapidly entering routine use in diagnostic surgical pathology. Reflex testing of all cases of colonic and endometrial adenocarcinoma is done in most comprehensive cancer centers in the US, and is starting to appear in Canada, with Manitoba being the most recent region to adopt reflex immunostaining (i.e. staining initiated by the pathologist signing out the case) as part of a comprehensive program to diagnose Lynch Syndrome. Although relatively uncommon, the endometriosis-associated ovarian carcinoma histotypes (endometrioid and clear cell carcinoma) have the same likelihood of MMR expression abnormalities as colonic and endometrial carcinoma, and we recommend inclusion of these cases in a reflex testing program. Recognition of Lynch syndrome has important implications for patient surveillance, which saves lives, and reflex testing of all newly diagnosed cancers of the types described above has been shown to lead to more diagnoses than reliance solely on any of the clinical criteria used to detect Lynch Syndrome.

In introducing new immunostains to the laboratory, there must be proper calibration of staining, and clinical validation through staining a series of cases of known MMR expression status. CIQC can help labs starting to do their staining by providing multiple samples with defined MMR expression status. Positive controls are essential for immunohistochemistry and for MMR immunostaining is relatively straight forward. There should be nuclear staining of some of the benign cells in any biopsy, no matter how small, and this positive internal control staining is essential for confident recognition of loss of staining.

The MMR proteins are fixation sensitive and expression is not detectable in poorly fixed tissues. As well, there is often a gradient seen in whole sections, with better fixed areas showing more intense staining. As a result, small tissue cores used for tissue microarray construction can be particularly challenging, as weakly stained areas of tumour may be sampled when building the tissue microarray. Nonetheless, performance of labs on this run was, overall, very good. Some labs had particularly nice staining and we would encourage you to compare your protocols to these labs, to see if any modifications to your staining might be possible to give improved staining, beyond "adequate" to "optimal". Note that because the MMR stains are typically used as a panel, deficiencies with one stain may be partially compensated for by good quality staining with another one of the markers. For example, poor MSH2 staining can be coped with in practice, if MSH6 staining is working well, given that MSH2 loss is seen in conjunction with MSH6 loss. As a result, it is possible that labs are accepting less than optimal staining for some MMR proteins, when relatively small modifications to their protocols could yield improved staining.

Definitions: In the case of MMR protein immunohistochemistry, nuclear staining = **expression (E)**, and is normal/indicative of non-mutant corresponding gene. **Absent (A)** staining of the tumour cell nuclei, with positive staining of non-tumour cells, is an abnormal result; 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 (F)** 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: Core 22, a weak expressing sample, showed positive staining of the stroma and tumour cells in only a minority of labs – this was considered optimal staining. Most labs had adequate or optimal results but the staining of the stromal cells could be improved. Four different clones were used. Clone M1 did appear to give false positive staining of core 4, for some labs. Only two labs used clone G168-728, and both had increased background staining (including strong staining of endothelial cells in one of these labs). Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Stromal staining weak in some cores
102	Adequate	Stromal staining weak in some cores
106	Optimal	No comment
107	Adequate	Stromal staining weak in some cores
111	Adequate	Stromal staining weak in some cores
112	Adequate	Stromal staining weak in some cores
114	Adequate	Generally weak staining
116	Inadequate	No staining
123	Optimal	No comment
125	Adequate	Stromal staining weak in some cores
144	Suboptimal	Interpretations changed at the time of review for cores 13, 25 and 22; false positive results in 4 and 13, while 25 was absent, based on review; in summary, two false positive results.
145	Suboptimal	High background staining resulted in high failure rate
149	Optimal	No comment
164	Adequate	Stromal staining weak in some cores
175	Adequate	False positive in core 4 plus high background in cytoplasm for some cores resulting in failure for core 6
181	Optimal	No comment
186	Adequate	High background with staining endothelials cells, resulting in failure
189	--	No slide submitted for review
191	Optimal	No comment
202	Optimal	No comment
210	Optimal	No comment

*Based on cIQc assessor consensus

PMS2: No "optimal" staining, as overall weaker or, for labs with stronger staining, some cytoplasmic background staining was observed. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Stromal staining weak in some cores
102	Adequate	Stromal staining weak in some cores
106	Adequate	Stromal staining weak in some cores
107	Adequate	Stromal staining weak in some cores
111	Adequate	Stromal staining weak in some cores
112	Adequate	Stromal staining weak in some cores
114	Adequate	Stromal staining weak in some cores
116	Adequate	Stromal staining weak in some cores
123	Adequate	Some cytoplasmic background staining
125	Adequate	Stromal staining weak in some cores
144	Adequate	Stromal staining weak in some cores
145	Inadequate	Very high background resulting in false negative results
149	Adequate	Stromal staining weak in some cores
164	Adequate	Stromal staining weak in some cores
175	Adequate	Stromal staining weak in some cores
181	Adequate	Stromal staining weak in some cores
186	Adequate	Stromal staining weak in many cores
189	--	No slide submitted for review
191	Adequate	Stromal staining weak in some cores
202	Adequate	Increase cytoplasmic background staining interferes with interpretation for some cores.
210	Adequate	Stromal staining weak in some cores

*Based on cIQc assessor consensus

MSH2: Endothelial staining was seen for some labs, interfering with interpretation of the normal internal control staining of stromal cells. This unusual staining pattern was only observed with the Ventana platform. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Inadequate	Weak staining of stromal cells and inappropriate staining of endothelial cells, with many failures.
102	Adequate	Weak staining of stromal cells in some cores
106	Optimal	No comment
107	Adequate	Some cytoplasmic background staining
111	Inadequate	Weak staining with too many failures
112	Adequate	Weak staining of stromal cells in some cores
114	Adequate	Cytoplasmic staining of endothelial cells interferes with interpretation
116	Adequate	Weak staining of stromal cells in some cores
123	Adequate	Some cytoplasmic background
125	Inadequate	Multiple failures due to weak staining of stromal cells
144	Optimal	No comment
145	Optimal	No comment
149	Optimal	No comment
164	Adequate	Cytoplasmic staining of endothelial cells interferes with interpretation
175	Optimal	No comment
181	Optimal	No comment
186	Inadequate	Core re-scored at assessment meeting. Strong staining of endothelial cells and weal stromal/tumour cell staining leading to multiple failures.
189	--	No slide submitted for review
191	Inadequate	Grossly overstained, with false positive results.
202	Adequate	Weak staining of stromal cells in some cores
210	Optimal	No comment

*Based on cIQc assessor consensus

MSH6: Notably, two of three labs using Clone 44 from BD Biosciences saw high cytoplasmic background, leading to inadequate staining. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Weak staining of stromal cells in some cores resulting in failures
102	Optimal	No comment
106	Optimal	No comment
107	Inadequate	High cytoplasmic background results in problems in interpretation, with multiple failures
111	Adequate	Overall slightly weak staining
112	Adequate	Weak staining of stromal cells in some cores resulting in failures
114	Adequate	Weak staining of stromal cells in some cores resulting in failures
116	Adequate	Weak staining of stromal cells in some cores
123	Optimal	No comment
125	Adequate	Weak staining of stromal cells in some cores resulting in failures
144	Adequate	Weak staining of stromal cells in some cores resulting in failures
145	Adequate	Some cytoplasmic background staining
149	Optimal	No comment
164	Adequate	Weak staining of stromal cells in some cores
175	Adequate	Some cytoplasmic background staining
181	Optimal	No comment
186	Adequate	Weak staining of stromal cells in some cores
189	--	No slide submitted for review
191	Inadequate	High cytoplasmic background results in problems in interpretation, with multiple failures
202	Adequate	Weak staining of stromal cells in some cores
210	Adequate	Weak staining of stromal cells in some cores

*Based on cIQc assessor consensus

The corrected Garrattograms are provided in Supplementary Figures 1 to 4. Supplementary Tables 1 to 4 contain reported staining protocols. Your regular participation in cIQc is greatly appreciated and we look forward to continually working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Figure S1. Revised Garrattogram after cIQc MLH1 IHC assessment.

Labs/Cores	101	102	106	107	111	112	114	116	123	125	144	145	149	164	175	181	186	189	191	202	210	REF
1	F	U	E	U	U	U	U	U	U	U	E	U	U	U	U	U	U	E	U	U	U	U
2	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
3	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
4	A	A	A	A	A	A	A	U	A	A	E	F	A	A	E	A	A	A	A	A	A	A
5	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
6	A	A	A	A	A	A	A	U	A	A	A	A	A	A	F	A	A	A	A	A	F	A
7	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
8	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
9	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
10	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
11	E	E	E	E	E	E	E	U	E	E	E	F	E	E	E	E	E	E	E	E	E	E
12	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
13	A	A	A	A	A	A	A	U	A	A	E	A	U	A	U	U	U	E	A	U	A	A
14	E	E	E	E	E	E	E	U	E	E	E	F	E	E	E	E	E	E	E	E	E	E
15	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	E	A	A	A	A
16	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
17	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
18	A	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	E	A	A	A	A
19	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
20	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
21	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
22	F	F	E	F	F	F	A	U	E	F	A	A	A	F	A	F	F	A	F	E	F	U
23	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
24	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
25	A	A	A	A	A	A	A	U	A	A	A	A	A	F	A	A	A	A	A	A	A	A
26	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
27	E	E	E	E	E	E	E	U	E	E	E	E	U	E	E	U	E	E	E	E	E	E
28	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	E	E	E	E	E	E	E	U	E	E	E	E	E	F	E	E	E	E	E	E	E	E
30	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E
31	A	A	A	A	A	A	A	U	A	A	A	A	A	A	F	A	A	A	A	A	A	A

E Expressed A Absent F Failed U Unsat

Figure S2. Revised Garrattogram after cIQc PMS2 IHC assessment.

Labs/Cores	101	102	106	107	111	112	114	116	123	125	144	145	149	164	175	181	186	189	191	202	210	REF
1	F	U	U	U	U	U	U	E	U	U	U	U	U	U	U	U	U	E	U	U	U	F
2	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E	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	F	A	A	A	A	F	A	A	A	A	A
5	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E	E	E	E
6	F	F	F	A	A	A	A	F	A	F	F	A	F	A	F	F	F	A	F	A	F	F
7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A
8	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E	E	E	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	E	U	E	E	E	E	E	E	U	E	E	E	U	E	E	E	E	E	E	E	U	U
11	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E	E	E	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	A	A	A	U	A	A	A	A	U	U	U	A	E	A	U	A	F
14	E	E	E	E	E	E	E	E	E	E	E	F	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	E	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	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
21	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
22	E	F	F	F	F	F	F	F	E	F	A	A	F	F	F	F	F	A	F	F	F	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
25	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	F	A	A	A	F	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	U	U	E	E	E	E	E	E	U	E	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	E	E	E	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E	E	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	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

E Expressed A Absent F Failed U Unsat

Figure S3. Revised Garrattogram after cIQc MSH2 IHC assessment.

Labs/Cores	101	102	106	107	111	112	114	116	123	125	144	145	149	164	175	181	186	189	191	202	210	REF
1	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	E	U	U	U	U
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
8	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	E	E	U	E	E	E	E	E	U	E	E	U	U	E	E	U	U	E	E	E	U	E
11	F	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	F	A	E	A	A	F
12	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
13	E	E	E	E	E	E	E	U	E	U	E	E	E	E	E	E	E	E	E	U	E	F
14	F	E	E	E	F	E	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	F
15	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
21	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
22	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
25	F	E	E	F	F	E	F	E	E	F	E	E	E	F	E	E	F	E	A	E	E	F
26	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
27	F	A	A	A	F	U	A	U	A	F	A	A	A	A	A	A	F	A	E	A	A	F
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	F	A	A	A	F	A	A	F	A	F	A	A	A	F	A	A	F	A	E	A	A	F
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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E

E Expressed A Absent F Failed U Unsat

Figure S4. Revised Garrattogram after cIQc MSH6 IHC assessment.

Labs/Cores	101	102	106	107	111	112	114	116	123	125	144	145	149	164	175	181	186	189	191	202	210	REF
1	F	E	E	U	U	U	A	U	U	U	U	U	U	U	U	U	U	E	U	U	U	F
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
8	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	E	U	E	U	U	E	E	E	E	U	E	U	U	U	E	E	U	E	E	E	U	E
11	A	A	A	F	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A
12	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
13	E	E	U	E	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	U	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	E	E	E	E	E	E	E	E
16	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
17	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
18	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
21	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
22	E	E	E	F	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
25	E	E	E	E	E	E	F	E	E	E	F	E	E	F	E	E	E	E	A	E	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	F	A	A	F	A	F	A	A	U	A	A	A	A	A	U	A	A	A	F	U	A	F
28	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
29	F	A	A	F	A	F	F	A	A	F	F	A	A	F	A	A	A	A	A	A	A	F
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	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E

E Expressed A Absent F Failed U Unsat

Table S1. Reported MLH1 staining protocols.

Lab	Clone	Dilution	Supplier	Antibody Lot #	Ab Incubation Time	Antigen Retrieval	Detection System	Enhancement
101	ES05	1:20	Novocastra	6023826	32 minutes	CC1 32 minutes	Optiview	Copper
102	ES05	1/150	DAKO	10077376	30" RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CUSO4
106	ESO5	1:50	Leica	6020041	60 min	115 C, 3 min, EDTA	MACH4 (polymer)	none
107	ES05	1:25	Leica/Novocastra	6023826	32 minutes	CCI - 56 minutes	Optiview DAB	None
111	G168-15	1/20	BD Pharmingen	3256964	32 min	CC1-64 min	Ultraview	Amplifier-DAB/Copper
112	G168-15	1:100 using Van Gogh Yellow diluent	BioCare Medical	120413	15 minutes	Bond Epitope Retrieval #2 for 30 min	Bond Polymer Refine	none
114	NCL-L-MLH1	1/50	Leica	6023826	16min	CC1 32min	Ventana optiview	copper
116	G168-15	1/40	BD PHARMINGEN	2138711	44 MIN	CC1 -48MIN	OPTIVIEW DAB VENTANA	YES
123	ES05	PREDILUTE	DAKO	10086338	20 MINUTES	HEAT WITH HIGH PH	MOUSE LINK/FLEX HRP	N/A
125	ES05	1/100	Dako	10085035	15 min	ER2-20	Bond Polymer Refine DAB	na
144	M1	Predilute	Ventana	D07345	56 min.	CC1	Optiview	Copper
145	G168-728	1/10	CELL MARQUE	1315706A	32 MINS	40 MINS CC1	XT OPTIVIEW DAB IHC V4	NO
149	ES05	RTU	Dako IR079	10080515 2015-03-31	20 min	high pH 98 C 20 min	DAB	EnVison Flex LINKER 15 min
164	M1	predilute	Ventana	D08621	32 minutes	ultraCC1/ 16minutes	Optiview	no
175	M1	Predilute	Roche	E0045	60	CC1	Optiview	Copper
181	ES05	1:50	DAKO	10079393	20 minutes	high pH	Envision Flex/mouse linker	NA
186	G168-728	1:50	Cell Marque	1315706A	15'	ER2/20'	Bond Refine Detection kit	None
189	M1	Predilute	Ventana	C10194	16min	CC1	OptiView DAB + Amp	Copper
191	ES05	1/10	Leica novocastra	6024040	32 min	CC1	ultraview	amplification A&B
202	ES05	1/10	BD PHARMAGEN	2138711	15 MIN	LEICA ER2 PH 9.5 20 MIN	LEICA REFINE DETECTION KIT	NONE
210	Clone E505	RTU	DAKO	10083784	30	Low	Envision Flex+	Mouse linker

Table S2. Reported PMS2 staining protocols.

Lab	Clone	Dilution	Supplier	Antibody Lot #	Ab Incubation Time	Antigen Retrieval	Detection System	Enhancement
101	EP51	1:15	Cedarlane	EL021804	32 minutes	CC1 32 minutes	Optiview	Copper
102	EP51	1/30	DAKO	10080012	30" RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CUSO4
106	MRQ-28	1:25	Cell Marque	1312701B	60 min	115 C, 3 min, EDTA	MACH4 (polymer)	none
107	EPR 3947	Pre-diluted	Ventana	1329701B	48 minutes	CCI - 40 minutes	Optiview DAB	Optiview Amplification
111	EPR3947	Pre-dilute	Ventana	1316203D	32 min	CC1-36 min	Ultraview	Amplifier/DAB-Copper
112	EP51	1:25	CEDARLANE	EL021804	15 minutes	Epitope Retrieval #2 for 40 min	Bond Polymer Refine	none
114	EP51	1/25	Epitomics	CJ071205	16min	CC1-32min	ventana optiview	Copper
116	EPR3947	RTU	Ventana	1329701C	60 min	CC1 64 min	Optiview DAB Ventana	yes
123	A16-4	1/50	BD BIOSCIENCES	3331643	30 MINUTES	HEAT WITH HIGH PH	MOUSE LINK /FLEX HRP	N/A
125	A16-4	1/100	BD Biosciences	3331643	15 min	ER2-20	Bond Polymer Refine DAB	na
144	EPR3947	predilute	Cell Marque	1316203D	64 min.	CC1	Optiview	Copper
145	MRQ28	1/5	CELL MARQUE	8050	32 MINS	40 MINS CC1	OPTIVIEW DAB IHC V4	NO
149	EP51	RTU	Dako IR087	10081525 2014-10-31	30 min	high pH 98 C 20 min	DAB	EnVison Flex LINKER 15 min
164	EPR3947	predilute	Ventana	1203404C	32 minutes	ultraCC1/ 56 minutes	Optiview	no
175	EPR3947	Predilute	Roche	1329701B	120	CC1	Optiview	Copper
181	EP51	1:50	DAKO	10078571	30 minutes	high pH	Envision Flex	NA
186	ERP3947	1:4	Cell Marque	1329701A	15'	ER2/20'	Bond Refine Detection syste	None
189	EPR3947	Predilute	Ventana	1220505C	24min	CC1	OptiView DAB + Amp	Copper
191	EPR3947	RTU	Roche	1329701B	32 min	CC2	Optiview	amplifier optiview
202	A16-4	1/25	BDPHARMAGEN	3331643	15 MIN	LEICA ER 2 PH 9.5 FOR 30 MIN	LEICA	NONE
210	EP51	RTU	DAKO	10085081	30	High	Envision flex+	N/A

Table S3. Reported MSH2 staining protocols.

Lab	Clone	Dilution	Supplier	Antibody Lot #	Ab Incubation Time	Antigen Retrieval	Detection System	Enhancement
101	G219-1129	1:1000	Cell Marque	1203409B	32 minutes	CC1 32 minutes	Optiview	Copper
102	FE11	1/150	CALBIOCHEM	D00133565	30" RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CUS04
106	25D12	1:75	Leica	6016520	60 min	115 C, 3 min, EDTA	MACH4 (polymer)	none
107	25D12	1:5	Leica/Novocastra	6023005	60 minutes	CCI - 60 minutes	Ultraview DAB	Ultraview Amplification
111	G219-1129	1/400	BD Pharmingen	2125584	32 min	CC1-64 min	iview	Blocker/DAB-Copper
112	FE11	1:50 renoir red diluent	BioCare Medical	120215	15 minutes	Epitope Retrieval #2 for 10 min	Bond Polymer Refine	none
114	G219-1129	1/1000	Cell Marque	1203409C	16 min	CC1 32min	ventana optiview	copper
116	G219-1129	1/3200	Cell Marque	1111801B	36 min	CC1- 40MIN	OPTIVIEW DAB VENTANA	NO
123	G219-1129	1/100	BD BIOSCIENCES	3200692	20 MINUTE	HEAT WITH HIGH PH	FLEX/HRP	N/A
125	25D12	predilute	Leica/Novocastra	26872	15 min	ER2-20	Bond Polymer Refine DAB	na
144	G129-1129	predilute	Cell Marque	1313004C	40 min	CC1	Optiview	copper
145	G219-1129	PRE-DILUTED	CELL MARQUE	16049	24 MINS	32 MINS CC1	OPTIVIEW DAB IHC V4	NO
149	FE11	RTU	Dako IR085	10082016 2014-10-31	20 min	high pH 98 C 20 min	DAB	None
164	G219-1129	predilute	Ventana	1203408C	8 minutes	ultraCC1/ 16 minutes	Optivew	no
175	G219-1129	Predilute	Roche	1313004D	32	CC1	Optiview	Copper
181	FE11	1:50	DAKO	10079031	20 minutes	high pH	Envision Flex	NA
186	G219-1129	1:1000	Cell Marque	1203409A	15'	ER2/20'	Bond Refine Detection kit	None
189	G219-1129	Predilute	Ventana	1218004B	12min	CC1	Optiview DAB	Copper
191	FE11	1/100	Sanbio(merck)	D00133565	32 min	CC1	Optiview	amplifier optiview
202	25D12	RTU	LEICA	25412	15 MIN	LEICAER 2 PH9.5 30 MIN	LEICA REFINE DETECTION KIT	NONE
210	FE11	RTU	Dako	10086886	20min	High	Envision flex+	Mouse linker

Table S4. Reported MSH6 staining protocols.

Lab	Clone	Dilution	Supplier	Antibody Lot #	Ab Incubation Time	Antigen Retrieval	Detection System	Enhancement
101	EP49	1:200	Cedarlane	CJ052301	32 minutes	CC1 32 minutes	Optiview	Copper
102	EP49	1/100	DAKO	10068079	30" RT	DAKO 3 IN 1 HIGH pH	DAKO FLEX	CUSO4
106	SP93	1:50	Cell Marque	1217103B	60 min	115 C, 3 min, EDTA	MACH4 (polymer)	none
107	44/MSH6	1:300	BD Biosciences	3200774	32 minutes	CCI - 60 minutes	Ultraview DAB	None
111	BC/44	Pre-dilute	BioCare Medical	20614	32 min	CC1-64 min	Ultraview	DAB/Copper
112	EP49	1:1500	Cedarlane	CJ052301L	15 minutes	Bond Epitope #2 for 40 min	Bond Polymer Refine	none
114	EP49	1/200	Epitomics	C1090101	16 min	CC1 32min	ventana optiview	copper
116	BC/44	1/200	BIOCARE MEDICAL	31313	36 MIN	CC1-32 MIN	OPTIVIEW DAB VENTANA	NO
123	EPR3945	1/300	ABCAM	GR129623-6	40 MINUTES	CC1-40 MINUTES	OPTVIEW (VENTANA)	COPPER/AMP(OPTVIEW)
125	44/MSH6	1/2000	BD Biosciences	11917	15 min	ER2-20	Bond Polymer Refine DAB	na
144	EP49	1:200	Cedarlane	CJ052301L	32 min.	CC1	Optiview	Copper
145	44	PRE-DILUTED	CELL MARQUE	6069	24 MINS	32 MINS CC1	OPTIVIEW DAB IHC V4	NO
149	EP49	RTU	Dako IR086	10081523 2014-09-30	20 min	high pH 98 C 20 min	DAB	None
164	44	predilute	Ventana	D08844	32 minutes	ultraCC1/ 16 minutes	Optiview	no
175	44	Predilute	Cell Marque	7040	32	CC1	Optiview	Copper
181	EP49	1:50	DAKO	10079041	30 minutes	high pH	Envision Flex	NA
186	BC/44	1:50	Biocare Medical	51313	15'	ER1/20'	Bond Refine Detection kit	None
189	44	Predilute	Ventana	D05353	12min	CC1	OptiView DAB + Amp	Copper
191	44	1/800	BDBiosciences	25975	32 min	CC1	ultraview	none
202	PU29	1/25	ABCAM	GR129623	15 MIN	LEICA ER2 PH9.5 FOR 40 MIN	LEICA REFINE DETECTION KIT	NONE
210	Clone EP49	RTU	DAKO	10086699	20	High	Envision flex+	N/A