



Assessors' report for cIQc Run 61

Assessors: B Gilks, J Garratt

Assessment performed: Vancouver General Hospital, July 16th 2016

Overview

A total of 28 labs participated in Run 61. Slides from Labs 115, 116 and 193 were not returned in time for the cIQc assessment meeting. Available slides from all other participating labs were blindly reviewed by cIQc assessors. Independent review led to occasional alteration of original self-reported results for discordant cores due to 1) an obvious data entry error or 2) a core score was deemed to be discordant between self-assessment and final cIQc review then re-classified.

MMR immunohistochemical staining continues to be of impressively good quality. All labs were considered to have either technically optimal or adequate staining by cIQc assessors, except for PMS2 staining by lab 189. General observations by assessors provided below, while specific feedback on each lab's staining is offered later in this report.

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.

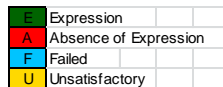
The MMR immunostains are always performed as part of a panel and the first Garratogram below shows nicely how MLH1 expression is not lost unless PMS2 expression is loss, with a similar relationship between MSH2 and MSH6. Because of this relationship, many labs do an initial screening for MMR loss by staining only for PMS2 and MSH6. This proficiency testing run illustrates two potential pitfalls that those using a two marker panel should be aware of, with respect to MSH6 expression:

1. **Weak MSH6 expression may be associated with absence of expression of MSH2 (e.g. core 3). If you are doing a two marker screen (i.e. MSH6 and PMS2) and there is very weak MSH6 expression, it would be prudent to proceed to MSH2 staining, so as to not miss any cases with MSH2 loss of expression.** This observation was also made in Run 52. In practice we are not seeing this as a common phenomenon, as only rare cases show this combination of MSH2 loss of expression and weak/focal MSH6 expression. At this point we do not know if any of these patients have Lynch syndrome or whether this expression pattern is a result of epigenetic changes.

2. **MSH6 weak expression can also be seen in some cases with MLH1 loss of expression (e.g. core 10).** This has been described in the literature and heterogeneity of MSH6 expression in these cases is attributable to microsatellite instability due to MLH1 or PMS2 loss, and not associated with an MSH6 germline mutation (Graham RP et al, Am J Surg Pathol 2015;39:1370-1376).

Apart from the two exceptional circumstances noted above, which users should be aware of, the interpretation of MMR immunostaining is straightforward. In our experience MMR staining works best on well-fixed small biopsy specimens (e.g. colonic or endometrial biopsies) and hysterectomy/colonic resection specimens are usable but show heterogeneity of staining because of variable fixation. The cores for this TMA are from large resection specimens, and between the small core size and variable antigen preservation, are not optimal specimens for MMR assessment, corresponding to the most challenging samples we will encounter in practice.

	MLH1	PMS2	MSH2	MSH6	Reference
1	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
2	[Grid]	[Grid]	[Grid]	[Grid]	MSH6
3	[Grid]	[Grid]	[Grid]	[Grid]	MSH2
4	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
5	[Grid]	[Grid]	[Grid]	[Grid]	PMS2
6	[Grid]	[Grid]	[Grid]	[Grid]	PMS2
7	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
8	[Grid]	[Grid]	[Grid]	[Grid]	MSH2
10	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
11	[Grid]	[Grid]	[Grid]	[Grid]	MSH2
12	[Grid]	[Grid]	[Grid]	[Grid]	PMS2
14	[Grid]	[Grid]	[Grid]	[Grid]	MSH6
15	[Grid]	[Grid]	[Grid]	[Grid]	MSH6
16	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
17	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
18	[Grid]	[Grid]	[Grid]	[Grid]	MSH6
19	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
21	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
22	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
23	[Grid]	[Grid]	[Grid]	[Grid]	MSH2
24	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
25	[Grid]	[Grid]	[Grid]	[Grid]	PMS2
26	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
27	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
31	[Grid]	[Grid]	[Grid]	[Grid]	PMS2
32	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
33	[Grid]	[Grid]	[Grid]	[Grid]	PMS2
34	[Grid]	[Grid]	[Grid]	[Grid]	MLH1
35	[Grid]	[Grid]	[Grid]	[Grid]	MSH6
38	[Grid]	[Grid]	[Grid]	[Grid]	MSH2



MLH1 Comments

MLH1	Score	Comments
101	Optimal	
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	
109	Adequate	Unusual granular nuclear staining (?nucleolar?) resulting in false +ve (expression) in cores 24 and 25. It is important to be aware of this pattern of "false positive" staining
110	Optimal	
111a	Optimal	
111b	Optimal	
112	Optimal	
114	Optimal	
115	No slides for review	
116	No slides for review	
124	Adequate	Weak staining with 3 failed cores due to no internal control staining
125	Optimal	
126	Optimal	
138	Optimal	
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	Unusual granular nuclear staining (?nucleolar?) resulting in false +ve (expression) in core 25. It is important to be aware of this pattern of "false positive" staining
189	Optimal	
193	No slides for review	
202	Adequate	False positive expression on core 22 attributable to high background staining
220	Adequate	Increased cytoplasmic background staining
222	Adequate	Unusual granular nuclear staining (?nucleolar?) resulting in false +ve (expression) in core 25. It is important to be aware of this pattern of "false positive" staining

	MLH1																												
Labs/Cores	101	102	103	106	107	109	110	111a	111b	112	114	115	124	125	126	138	141	144	145	149	175	181	186	189	193	202	220	222	
1	A	U	A	A	A	U	A	A	A	E	U	U	U	A	U	U	U	A	A	U	A	U	A	A	U	U	A	U	
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
3	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	E	E	E	E	E	E	E	E
4	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	
5	F	E	E	E	E	E	E	E	E	E	E	A	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	U	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	
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	
8	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	
10	A	A	A	A	A	A	A	A	A	A	A	U	F	A	A	A	U	A	A	A	U	A	A	A	F	A	A	A	
11	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	
12	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	F	E	
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	
15	E	E	E	E	E	E	E	E	E	E	F	F	F	F	E	E	E	E	E	E	E	E	F	F	F	E	E	F	
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	
17	U	A	U	U	U	U	A	U	A	A	A	A	A	A	A	A	A	A	U	A	U	A	U	U	A	U	U	A	
18	E	E	E	E	E	E	E	E	E	E	E	A	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	
19	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
21	A	A	A	A	A	A	A	A	A	A	A	U	U	U	U	U	A	U	A	A	A	A	A	A	F	A	A	A	
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	A	
23	E	E	E	E	E	E	E	E	E	E	E	F	A	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	
24	A	A	A	A	U	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	A	A	A	E	
25	A	A	A	A	U	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	U	A	E	A	A	U	A	A	
26	A	A	A	A	A	A	A	A	A	A	A	U	U	U	A	U	U	U	A	U	A	A	A	A	A	A	A	A	
27	A	A	A	A	A	A	A	A	A	A	A	F	F	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	
31	E	E	U	E	U	E	E	U	E	U	E	E	E	E	E	E	E	E	U	U	U	E	U	E	E	U	E	E	
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	F	A	A	A	
33	A	A	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
34	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	A	A	A	
35	U	E	E	E	U	E	E	U	E	E	E	E	E	E	E	E	E	E	U	E	U	E	U	U	F	U	U	E	
38	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	

- E Expression
- A Absence of Expression
- F Failed
- U Unsatisfactory

MLH1 Descriptive Statistics									
Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	30	90	27	27/27 (100%)	1	1	1	1	1
102	30	96.67	29	29/29 (100%)	1	1	1	1	1
103	30	90	27	27/27 (100%)	1	1	1	1	1
106	30	96.67	29	29/29 (100%)	1	1	1	1	1
107	30	80	24	24/24 (100%)	1	1	1	1	1
109	30	86.67	26	26/26 (100%)	1	1	1	1	1
110	30	100	30	30/30 (100%)	1	1	1	1	1
112	30	96.67	29	28/29 (97%)	0.94	1	1	0.93	0.93
114	30	93.33	28	28/28 (100%)	1	1	1	1	1
115	30	66.67	20	17/20 (85%)	0.9	0.8	0.82	0.89	0.7
124	30	73.33	22	21/22 (95%)	1	0.92	0.91	1	0.91
125	30	90	27	27/27 (100%)	1	1	1	1	1
126	30	86.67	26	26/26 (100%)	1	1	1	1	1
138	30	90	27	27/27 (100%)	1	1	1	1	1
141	30	86.67	26	26/26 (100%)	1	1	1	1	1
144	30	93.33	28	28/28 (100%)	1	1	1	1	1
145	30	90	27	27/27 (100%)	1	1	1	1	1
149	30	90	27	27/27 (100%)	1	1	1	1	1
175	30	73.33	22	22/22 (100%)	1	1	1	1	1
181	30	96.67	29	29/29 (100%)	1	1	1	1	1
186	30	86.67	26	25/26 (96%)	0.93	1	1	0.92	0.92
189	30	90	27	27/27 (100%)	1	1	1	1	1
193	30	70	21	21/21 (100%)	1	1	1	1	1
202	30	83.33	25	24/25 (96%)	0.92	1	1	0.92	0.92
220	30	90	27	27/27 (100%)	1	1	1	1	1
222	30	93.33	28	27/28 (96%)	0.93	1	1	0.93	0.93
111a	30	90	27	27/27 (100%)	1	1	1	1	1
111b	30	96.67	29	29/29 (100%)	1	1	1	1	1

MLH1 PROTOCOLS											
Labs/Fields	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	Heat (cc1)	32	ES05	1:50	Leica	6023826	32	optiview	no	copper	cab
102	Dako TRS High pH	10/20/10	ES05	1:80	DAKO	10105399	30*RT	DAKO FLEX+	YES	CUSO4	DAB+
103	CC1	56 MINS	M1	PRE	VENTANA	F02382	48 MINS	OPTIVIEW	No	Yes	DAB
106	Microwave pressure cooker	30 min	ES05	1:85	Leica	6038153	45 min	MACH4	no	no	DAB
107	cc1	64	ES05	1:20	Novocastra	6038153	32	Optiview DAB	N	Y	DAB
109	HIER high Ph(cc1)	32 min	M1	RUT	ROCHE	E06127	16 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High pH	20 min @97 C	ES05	1:50	DAKO	10105399	20 min	Dako Envision Flex	Y	N	DAB
111a	CC1	48 MIN	G168-15	1/25	B.D.PHARMINGEN	3256964	32 MIN	OPTIVIEW	N	COPPER	DAB
111b	CC1	48 MIN	G168-15	1:50	Biocare	42616	32 MIN	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2	25 minutes	ES05	1:100	Leica (Novocastra)	6023826	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	32	ES05	1/50	Leica	6023826	44	Optiview	N	Copper	DAB
115	envision flex high pH	30 mins	ES05	RTU	Dako	10107799	20 mins	envision flex	N	N	envision flex substrate working solution
116	CC1	48 MIN	G168-15	1/80	BD PHARMINGEN	2138711	44 MIN	OPTIVIEW DAB	Y	COPPER	DAB
124	CC1	64 min	ES05	1/100	Leica	6025230	16	Optiview	O	N	Dab
125	Dako EnV FLEX TRS high pH	30	ES05	RTU	DAKO	10103827	30	Dako EnV FLEX	Y	N	DAB
126	Microwave pressure cooker, with citrate buffer ph 6.01	36 minutes	ES05	1:50	Dako	10110535	30 minutes	Quanto	no	no	DAB +
138	HIER - EDTA	20	ES05	RTU	Dako	10103827	20	Polymer	Y	N	DAB
141	HIER	20	ES05	1:50	Dako	10105399	20	Polymer	Y	N	DAB
144	CC1	56 min	ES05	1:25	Novocastra	6025230	32 min	Optiview	No	Yes, Copper	DAB
145	CC1	56	G168-15	1/50	BIOCARE	20215	32	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	ES05	RTU	Dako	10107172	30	EnVision Flex	No	No	DAB
175	HIER	64	M1	Pre dilute	Roche	F08049	16	Polymer	n	y	DAB
181	HIER	20 minutes	ES05	1:50	DAKO	10105399	20 minutes	HRP-Polymer	Y	N	DAB
186	HIER	20 MIN.	G168-728	1:50	CELL MARQUE	1313506G 1313506G	15 MIN.	POLYMER	N	N	DAB
189	CC1	64	M1	pre-dilute	Ventana	unknown	16	ultraView	N	N	DAB
193	Low pH Omnis	30 min	ES05	RTU	Dako	10101385	30 min	Envision Flex	Yes	No	DAB
202	Leica ER2 citrate Buffer 9.5	20min	es05	1/10	BD Pharmagen	5215664	15 min	Refine Detection kit Leica	no	no	dab
220	CC1	72	ES05	1/25	Dako	10104116	1hr	OptiView	N	N	DAB
222	CC1	90min	M1	RTU	Roche	F08049	4min	Optiview DAB	Y	Copper	DAB

PMS2 Comments

PMS2	Score	Comments
101	Optimal	
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
115	No slides for review	
116	No slides for review	
124	Optimal	
125	Optimal	
126	Optimal	
138	Optimal	
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Optimal	
189	Suboptimal	Too many (6) failed cores due to weak staining
193	No slides for review	
202	Adequate	High background cytoplasmic staining
220	Adequate	High background cytoplasmic staining
222	Optimal	Unusual granular cytoplasmic background staining, but did not interfere with interpretation

	PMS2																										
Labs/Cores	101	102	103	106	107	109	110	111	112	114	115	124	125	126	138	141	144	145	149	175	181	186	189	193	202	220	222
1	A	U	A	A	A	A	A	A	A	U	U	U	A	U	U	A	A	A	U	U	U	A	A	A	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	E	E	E	E	E
3	E	E	U	E	E	U	U	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
5	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	F	A	A
6	A	A	A	A	A	A	A	A	A	A	U	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	U
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
8	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	E	E	E	E	E	E	E
10	U	A	A	A	A	A	A	A	A	A	U	A	F	U	A	U	A	A	A	U	A	F	A	A	A	A	A
11	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
12	A	A	A	A	A	A	A	A	A	A	A	U	U	U	U	A	A	A	A	A	A	A	A	F	F	A	A
14	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
15	U	E	F	E	E	F	E	F	E	E	F	F	E	E	U	E	E	E	E	F	E	E	F	E	E	F	E
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
17	U	A	U	U	U	U	U	U	U	U	A	A	A	A	A	A	A	U	A	U	A	A	U	U	A	A	A
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
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
21	A	A	A	A	A	A	A	A	A	A	U	U	U	U	U	U	U	A	A	A	A	A	A	A	A	U	A
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
23	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
24	A	A	U	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A
25	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
26	A	U	A	A	A	A	A	A	A	A	U	U	U	A	U	U	U	A	A	A	A	A	A	A	A	U	A
27	U	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A
31	E	E	U	E	E	U	U	U	U	E	E	E	E	E	E	E	E	U	U	U	E	U	E	E	U	E	E
32	A	A	A	A	A	A	A	A	A	A	U	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A
33	A	A	A	A	A	A	A	A	A	A	E	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A
34	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A
35	U	E	U	E	U	U	U	U	E	E	A	E	E	E	F	E	E	E	E	U	E	E	U	U	E	E	E
38	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 Expression
- A Absence of Expression
- F Failed
- U Unsatisfactory

PMS2 Descriptive Statistics									
Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	30	80	24	24/24 (100%)	1	1	1	1	1
102	30	93.33	28	28/28 (100%)	1	1	1	1	1
103	30	76.67	23	23/23 (100%)	1	1	1	1	1
106	30	96.67	29	29/29 (100%)	1	1	1	1	1
107	30	93.33	28	28/28 (100%)	1	1	1	1	1
109	30	76.67	23	23/23 (100%)	1	1	1	1	1
110	30	86.67	26	26/26 (100%)	1	1	1	1	1
111	30	83.33	25	25/25 (100%)	1	1	1	1	1
112	30	93.33	28	28/28 (100%)	1	1	1	1	1
114	30	93.33	28	28/28 (100%)	1	1	1	1	1
115	30	60	18	16/18 (89%)	0.91	0.86	0.91	0.86	0.77
124	30	83.33	25	25/25 (100%)	1	1	1	1	1
125	30	86.67	26	26/26 (100%)	1	1	1	1	1
126	30	83.33	25	25/25 (100%)	1	1	1	1	1
138	30	66.67	20	20/20 (100%)	1	1	1	1	1
141	30	90	27	27/27 (100%)	1	1	1	1	1
144	30	93.33	28	28/28 (100%)	1	1	1	1	1
145	30	93.33	28	28/28 (100%)	1	1	1	1	1
149	30	93.33	28	28/28 (100%)	1	1	1	1	1
175	30	76.67	23	23/23 (100%)	1	1	1	1	1
181	30	96.67	29	29/29 (100%)	1	1	1	1	1
186	30	93.33	28	28/28 (100%)	1	1	1	1	1
189	30	73.33	22	22/22 (100%)	1	1	1	1	1
193	30	86.67	26	26/26 (100%)	1	1	1	1	1
202	30	90	27	27/27 (100%)	1	1	1	1	1
220	30	83.33	25	25/25 (100%)	1	1	1	1	1
222	30	96.67	29	29/29 (100%)	1	1	1	1	1

PMS2 PROTOCOLS											
Labs/Fields	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	heat (cc1)	32	ep51	1:15	epitomics	EN031503	32	optiview	no	copper	dab
102	Dako TRS High pH	10/20/10	EP51	1:20	DAKO	10107094	30*RT	DAKO FLEX+	YES	CUSO4	DAB+
103	CC1	64 MINS	EPR3947	PRE	CELL MARQUE	1506813B	60mins	OPTIVIEW + LINKER	Yes	Yes	DAB
106	Microwave pressure cooker	30 min	MRQ-28	1:15	Cell marque	1430403F	45 min	MACH4	no	no	DAB
107	Decloaking - HEX TRS High pH 9.0	120 C - 30 sec	A16 - 4	1:200	BD Pharmingen	5174534	30	Flex + 30	N	N	DAB
109	HIER high pH(CC1)	64 MIN	EPR3947	RTU	CELL MARQUE	1417001B	20 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High pH	20 min @97 C	EP51	1:50	DAKO	10107094	30 min	Dako Envision Flex	N	N	DAB
111	CC1	48 MIN	EP51	1/100	DAKO	10112572	32 MIN	OPTIVIEW	Y	COPPER	DAB
112	BOND Epitope Retrieval 2	30 minutes	EP51	1:75 using background reducing diluent	DAKO	10109389	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	64	EP51	1/25	Epitomics	EM022410	64	Optiview	N	Copper	DAB
115	envision flex high pH	30 mins	EP51	RTU	Dako	10108822	30 mins	envision flex	N	N	envision flex working solution
116	CC1	64 min	EPR3947	RTU	VENTANA	1506813 C	60 MIN	OPTIVIEW	Y	COPPER	DAB
124	CC1	56 min	EP51	1/40	Dako	10108546	32	Optiview	N	N	Dab
125	Dako EnV FLEX TRS high pH	30	A16-4	1/75	BD BIOSCIENCES	5051647	25	Dako EnV FLEX	Y	N	DAB
126	Microwave pressure cooker, with citrate buffer pH 6.01	36 minutes	EP51	1:25	Dako	10110537	30 minutes	Quanto	No	NO	DAB +
138	HIER - EDTA	20	EP51	RTU	Dako	10107620	30	Polymer	N	N	DAB
141	HIER	20	EP51	1:50	Dako	10107094	30	Polymer	Y	N	DAB
144	CC1	64 min	EPR3947	Pre-Dilute	Cell Marque	1532405B	60 min	Optiview	No	Yes, Copper	DAB
145	CC1	64	EPR3947	1/15	CELL MARQUE	1506813A	44	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	EP51	RTU	Dako	10108854	20	EnVision Flex	Yes	No	DAB
175	HIER	64	EPR3947	Pre dilute	Roche	1532405B	32	OPTI-DAB POLYMER	Y	Y	DAB
181	HIER	20 minutes	EP51	1:50	DAKO	10107094	30 minutes	HRP-polymer	N	N	DAB
186	HIER	20 min	EPR3947	1:4	Cell Marque	1506813A	15 min	POLYMER	N	N	DAB
189	CC1	64	EPR3947	pre-dilute	Cell Marque	unknown	32	ultraView	Y	N	DAB
193	Hi pH Omnis	30 min	EP51	RTU	Dako	10102813	30 min	Envision Flex	Yes	No	DAB
202	Leica ER2 citrate Buffer 9.5	30 min	a16-4	1/25	BD Pharmagen	5051647	15 min	Refine Detection kit Leica	no	no	dab
220	CC1	64	EP51	1/40	Dako	10108546	1h8min	Optiview	N	N	DAB
222	CC1	90 min	EPR3947	RTU	Roche	1506813E	44 min	DAB Optiview	Y	Copper	DAB

MSH2 Comments

MSH2	Score	Comments
101	Adequate	4 failed cores due to lack of internal control staining
102	Optimal	
103	Optimal	
106	Optimal	
107	Optimal	
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	
115	No slides for review	
116	No slides for review	
124	Optimal	
125	Adequate	2 failed results and one false –ve (absence) on core 27
126	Optimal	Beautiful staining!
138	Optimal	
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Optimal	
189	Adequate	3 failed cores due to lack of internal control staining
193	No slides for review	
202	Optimal	
220	Optimal	
222	Optimal	

	MSH2																											
Labs/Cores	101	102	103	106	107	109	110	111	112	114	115	124	125	126	138	141	144	145	149	175	181	186	189	193	202	220	222	
1	U	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	U	U	E	E	U	U	E	
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
3	A	U	A	A	A	A	U	A	A	A	U	U	A	A	A	A	A	A	A	A	A	A	A	U	A	A	A	
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	
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	
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E	E	U	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	E	E	E	E	E	
8	A	A	A	A	A	A	A	A	A	A	A	A	A	U	U	A	A	A	A	A	A	A	U	A	A	A	A	
10	U	E	E	E	E	E	E	E	E	E	E	E	E	U	E	U	E	E	E	U	E	U	E	E	E	E	E	
11	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	
12	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	E	E	E	E	U	E	E	E	E	
14	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	
15	F	E	E	E	F	F	E	A	E	F	A	F	F	E	F	E	E	F	E	F	E	F	F	F	F	F	F	
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	
17	U	U	U	E	E	U	U	E	E	E	U	U	E	E	E	E	U	E	E	U	E	E	U	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	E	E	E	E	E	
19	E	E	E	E	E	E	E	E	E	E	A	E	E	E	U	U	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	U	U	E	E	U	U	E	E	E	U	E	E	E	U	
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	
23	F	A	A	A	A	A	A	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A	F	F	A	A	A	
24	E	U	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	
25	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	
26	U	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	U	E	E	E	E	U	E	E	E	U	
27	F	E	E	E	E	E	E	E	E	E	A	E	A	E	E	E	E	E	E	E	E	E	E	F	E	E	E	
31	E	U	U	E	E	E	U	E	U	E	E	E	E	E	U	E	E	U	U	U	E	U	E	U	U	E	E	
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	
33	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	
34	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	
35	F	U	U	E	F	F	U	E	E	E	U	U	U	E	F	E	U	F	E	U	F	F	F	U	F	F	F	
38	F	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	

- E Expression
- A Absence of Expression
- F Failed
- U Unsatisfactory

MSH2 Descriptive Statistics									
Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	30	70	21	21/21 (100%)	1	1	1	1	1
102	30	80	24	24/24 (100%)	1	1	1	1	1
103	30	90	27	27/27 (100%)	1	1	1	1	1
106	30	100	30	30/30 (100%)	1	1	1	1	1
107	30	93.33	28	28/28 (100%)	1	1	1	1	1
109	30	86.67	26	26/26 (100%)	1	1	1	1	1
110	30	86.67	26	26/26 (100%)	1	1	1	1	1
111	30	100	30	29/30 (97%)	1	0.96	0.83	1	0.89
112	30	96.67	29	29/29 (100%)	1	1	1	1	1
114	30	96.67	29	29/29 (100%)	1	1	1	1	1
115	30	86.67	26	23/26 (88%)	1	0.86	0.57	1	0.66
124	30	86.67	26	26/26 (100%)	1	1	1	1	1
125	30	90	27	26/27 (96%)	1	0.96	0.8	1	0.87
126	30	86.67	26	26/26 (100%)	1	1	1	1	1
138	30	63.33	19	19/19 (100%)	1	1	1	1	1
141	30	80	24	24/24 (100%)	1	1	1	1	1
144	30	93.33	28	28/28 (100%)	1	1	1	1	1
145	30	83.33	25	25/25 (100%)	1	1	1	1	1
149	30	93.33	28	28/28 (100%)	1	1	1	1	1
175	30	76.67	23	23/23 (100%)	1	1	1	1	1
181	30	93.33	28	28/28 (100%)	1	1	1	1	1
186	30	83.33	25	25/25 (100%)	1	1	1	1	1
189	30	73.33	22	22/22 (100%)	1	1	1	1	1
193	30	73.33	22	22/22 (100%)	1	1	1	1	1
202	30	86.67	26	26/26 (100%)	1	1	1	1	1
220	30	90	27	27/27 (100%)	1	1	1	1	1
222	30	86.67	26	26/26 (100%)	1	1	1	1	1

MSH2 PROTOCOLS											
Labs/Fields	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	heat (cc1)	32	G219-1129	1:200	Cell Marque	1313003B	32	optiview	no	copper	dab
102	Dako TRS High pH	10/20/10	FE11	1:40	Dako	10102927	30"RT	DAKO FLEX+	YES	CUS04	DAB+
103	CC1	56 MINS	G219-1129	PRE	CELL MARQUE	1417104F	32 MINS	OPTIVIEW	No	Yes	DAB
106	Microwave pressure cooker	30 min	FE311	1:50	Dako	10111672	45 min	MACH4	no	n	DAB
107	cc1	32	G219-1129	1:200	Cell Marque	1334002A	32	Optiview DAB	N	Y	DAB
109	HIER high pH (CC1)	32 MIN	G219-1129	RTU	CELL MARQUE	1417104C	8 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High pH	20 min @97 C	FE11	1:150	DAKO	10106449	20 min	Dako Envision Flex	Y	N	DAB
111	CC1	40 MIN	G219-1129	1/600	CELL MARQUE	1616010A	32 MIN	OPTIVIEW	Y	COPPER	DAB
112	BOND Epitope Retrieval 2	30 minutes	FE11	1:100	DAKO	10109387	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	32	G219-1129	1/100	Cell Marque	1505808B	32	Optiview	N	Copper	DAB
115	envision flex high pH	30 mins	FE11	RTU	Dako	10107056	20 mins	envision flex	N	N	envision flex substrate working solution
116	CC1	40 MIN	G219-1129	1/400	CELL MARQUE	3003 B	48 MIN	OPTIVIEW	N	COPPER	DAB
124	CC1	24 min	G219-1129	prÃ©diluÃ©	Cellmarque	1417104G	16	Dab	n	n	Dab
125	Dako EnV FLEX TRS high pH	30	25D12	1/50	Novocastra/Leica	6035858	30	Dako EnV FLEX	Y	N	DAB
126	Microwave pressure cooker, with citrate buffer ph 6.01	36 minutes	FE11	1:75	Dako	10109387	30 minutes	Quanto	No	NO	DAB +
138	HIER - EDTA	20	FE11	RTU	Dako	10100791	20	Polymer	Y	N	DAB
141	HIER	20	FE11	1:150	Dako	10106449	20	Polymer	Y	N	DAB
144	CC1	40 min	G219-1129	Pre-Dilute	Cell Marque	1417104G	16 min	Optiview	No	Yes, Copper	DAB
145	CC1	40	G219-1129	1/400	CELLMARQUE	1313003A	24	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	FE11	RTU	Dako	1010756	20	EnVision Flex	Yes	No	DAB
175	HIER	32	G219-1129	Pre dilute	Roche	1529502C	16	Polymer	n	y	DAB
181	HIER	20 minutes	FE11	1:150	Dako	10106449	20 minutes	HRP-Polymer	Y	N	DAB
186	HIER	20 MIN.	G219-1129	1:200	Cell Marque	13334002F	15 MIN	POLYMER	N	N	DAB
189	CC1	32	G219-1129	pre-dilute	Cell Marque	unknown	16	ultraView	N	N	DAB
193	High pH Omnis	30 min	FE11	RTU	Dako	10100791	30 min	Envision Flex	No	No	DAB
202	Leica ER2 citrate Buffer 9.5	30 min	25d12	1//50	Leica	603917	15 min	Refine Detection kit Leica	no	no	DAB
220	CC1	32	G219-1129	prediluted	Cell Marque	1505808E	32	Optiview	N	N	DAB
222	CC1	90min	G219-1129	RTU	Roche	1505808E	12min	DAB Optiview	N	Copper	DAB

MSH6 Comments

MSH6	Score	Comments
101	Optimal	
102	Optimal	
103	Optimal	
106	Optimal	
107	Adequate	Too many failed cores due to high cytoplasmic background
109	Optimal	
110	Optimal	
111	Optimal	
112	Optimal	
114	Optimal	Weak expression in cores 11 and 38, which are negative in most labs (cases of MSH2 loss)
115	No slides for review	
116	No slides for review	
124	Optimal	
125	Optimal	
126	Optimal	
138	Optimal	
141	Optimal	
144	Optimal	
145	Optimal	
149	Optimal	
175	Optimal	
181	Optimal	
186	Adequate	High cytoplasmic background staining
189	Adequate	Weak staining with too many failed cores
193	No slides for review	
202	Adequate	Weak positive staining of cores 8, 27, 38 (all cases with MSH2 loss)
220	Optimal	
222	Optimal	

MSH6 PROTOCOLS											
Labs/Fields	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	heat (cci)	32	EP49	1:100	Epitomics	EN020910	32	optiview	no	copper	dab
102	Dako TRS High pH	10/20/10	EP49	1:100	DAKO	10100346	30"RT	DAKO FLEX	NO	CUSO4	DAB+
103	CC1	64	44	PRE	VENTANA	F01005	60 MINS	OPTIVIEW + LINKER	No	Yes	DAB
106	Microwave pressure cooker	30 min	SP93	1:15	Cell marque	1526102B	45 min	MACH4	no	no	DAB
107	cc1	60	44/MSH6	1:300	BD	4339757	32	Ultraview DAB	N	Y	DAB
109	HIER high pH (CC1)	64 MIN	44	RTU	ROCHE	E05684	20 MIN	OPTIVIEW	N	Y	DAB
110	DAKO PT High pH	20 min @97 C	EP49	1:200	DAKO	10106453	30 min	Dako Envision Flex	N	N	DAB
111	CC1	48 MIN	SP93	1/100	CELL MARQUE	1512802D	32 MIN	OPTIVIEW	N	COPPER	DAB
112	BOND Epitope Retrieval 2	40 minutes	EP49	1:1500	Epitomics	EL120401	30 minutes	BOND Polymer Refine	none	none	DAB
114	CC1	64	EP49	1/200	Epitomics	C1090707	32	Optiview	N	Copper	DAB
115	envision flex high pH	30 mins	EP49	RTU	Dako	10107433	20 mins	envision flex	N	N	envision flex substrate working solution
116	CC1	32 MIN	70814	1/400	BIOCARE MEDICAL	70814	48 MIN	OPTIVIEW DAB	N	COPPER	DAB
124	CC1	64 min	EP49	1/50	Dako	10100346	32	Optiview	N	N	Dab
125	Dako EnV FLEX TRS high pH	30	44/MSH6	1/500	BD BIOSCIENCES	5225898	60	Dako EnV FLEX	Y	N	DAB
126	Microwave pressure cooker, with citrate buffer pH 6.01	36 minutes	EP49	1:75	Dako	10109532	30 minutes	Quanto	No	NO	DAB +
138	HIER - EDTA	20	EP49	RTU	Dako	10099688	20	Polymer	N	N	DAB
141	HIER	20	EP49	1:200	Dako	10106453	30	Polymer	Y	N	DAB
144	CC1	32 min	EP49	1:100	Cedarlane	EL061801	32 min	Optiview	No	Yes, Copper	DAB
145	CC1	32	44	1/300	CELLMARQUE	1313501A	16	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	PT Link high pH	20 min at 97 C	EP49	RTU	Dako	10102086	20	EnVision Flex	No	No	DAB
175	HIER	64	44	1 in 100	Cell Marque	1112208C	32	polymer	y	y	DAB
181	HIER	20 minutes	EP49	1:200	DAKO	10106453	30 minutes	HRP-Polymer	N	N	DAB
186	HIER	20 MIN	BC/44	1:50	Biocare Medical	50815	15 MIN	POLYMER	N	N	DAB
189	CC1	64	44	pre-dilute	Ventana	unknown	16	ultraView	N	N	DAB
193	HIER high pH Omnis	30 min	EP49	RTU	Dako	10102086	20 min	Envision Flex	No	No	DAB
202	Leica ER2 citrate Buffer 9.5	40	pu29	1/25	abcam	gr189185	15 min	Refine Detection kit Leica	no	no	DAB
220	CC1	32	EP49	1/50	Dako	10100348	28min	OptiView	N	N	DAB
222	CC1	90min	44	RTU	Rochr	F07076	16min	Optiview DAB	Y	Copper	DAB