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## Assessors' report for cIQc Run 52: MMR Immunohistochemistry

Assessors: B Gilks and B Clarke

Assessment performed on Friday, November 27<sup>th</sup>, 2015 at Toronto General Hospital

*Note: Apologies for the lateness in issuing this report! We realized after the assessment meeting that we should get MLH1 promoter methylation studies done on some of the case, as an aid to interpretation, and between this and the Christmas break, this assessment report was significantly delayed.*

### **Overview**

A total of 35 labs participated in Run 52. Slides from Labs 189 and 217 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.

The trend of improved MMR immunohistochemical staining continues to be observed. All labs were considered to have either technically optimal or adequate staining by cIQc assessors, and results were excellent, overall. 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 some heterogeneity of MLH1 expression associated with MLH1 promoter methylation. Cores 9, 10 and 23 were from tumors with promoter methylation, while cores 27 and 39 were from tumors with low level promoter methylation. Cores 9, 10 and 23 showed uniform absence of staining for MLH1, in all but one instance (core 23 showed MLH1 expression on the slide from lab 217; we were not able to validate whether this was an interpretation as the slide was not available for review). In contrast, cores 27 and 39, where there was low-level MLH1 promoter methylation, showed considerable variability in immunostaining results, an expected consequence of the partial methylation of the promoter. We considered either expression or absence of expression to be correct results for these two cores, 27 and 29, as the staining would depend on the cells present for evaluation on each slide, and with the heterogeneity of MLH1 expression that would be anticipated to result from low level promoter methylation one could see either expression or absence of expression for any given slide. Core 30 was a tumor with MLH1 loss and no promoter methylation; all labs had the correct result for this core. **SUMMARY: Excluding cores 27 and 39, from tumors with low level MLH1 promoter methylation where there were variable MLH1 staining results, the staining results for MLH1 were near perfect with a single "false negative" result i.e. staining present in a case where absence of staining is the expected result, and no false positive results.**



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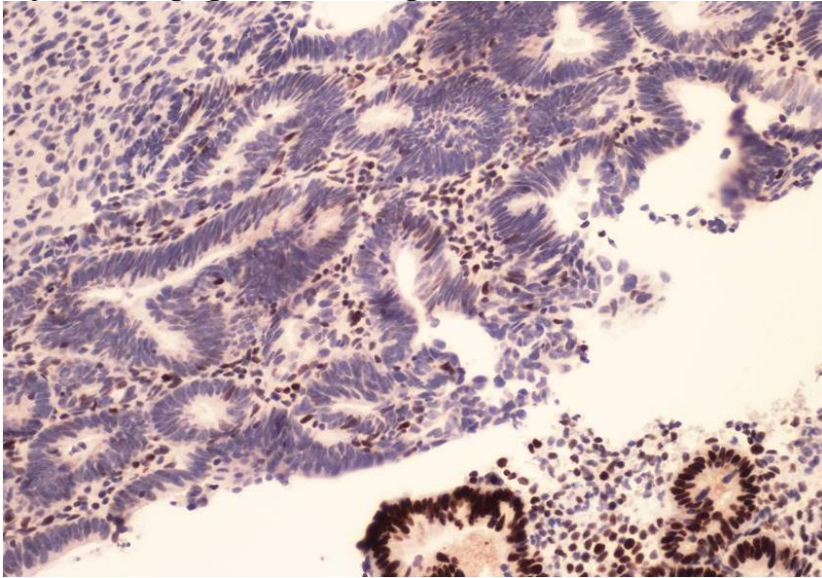
**PMS2:** PMS2 staining, which had been challenging in previous runs, were excellent. There were two cores from tumors with isolated absence of PMS2 expression (cores 2 and 36) and all labs had correct results for those cores. PMS2 is also lost in cases with loss of MLH1 expression (e.g. core 30, which all labs assessed as showing absence of PMS2 staining). PMS2 expression was variable in the five cores with MLH1 promoter methylation, just as MLH1 expression was variable. Note that PMS2 expression did not correlate perfectly with MLH1 expression in a given case; Isolated PMS2 loss could be seen in cases with heterogeneous MLH1 expression due to MLH1 promoter methylation (e.g. core 27), which has been described previously (Rosty, Christophe, et al. "Germline mutations in PMS2 and MLH1 in individuals with solitary loss of PMS2 expression in colorectal carcinomas from the Colon Cancer Family Registry Cohort." *BMJ open* 6.2 (2016): e010293; PMID [26895986](#)). It is now well established that isolated absence of PMS2 may be a result of MLH1 promoter methylation, or less commonly, mutation in MLH1, and this should be a consideration in the investigation of patients whose tumors show isolated absence of PMS2 expression. **SUMMARY: Excluding cores 27 and 39, with low level MLH1 promoter methylation, where there were variable PMS2 staining results, the staining results for PMS2 showed 100% sensitivity and 100% specificity, if failed/uninterpretable results were excluded.**

**MSH2: MSH 2 staining results showed 100% sensitivity and specificity.**

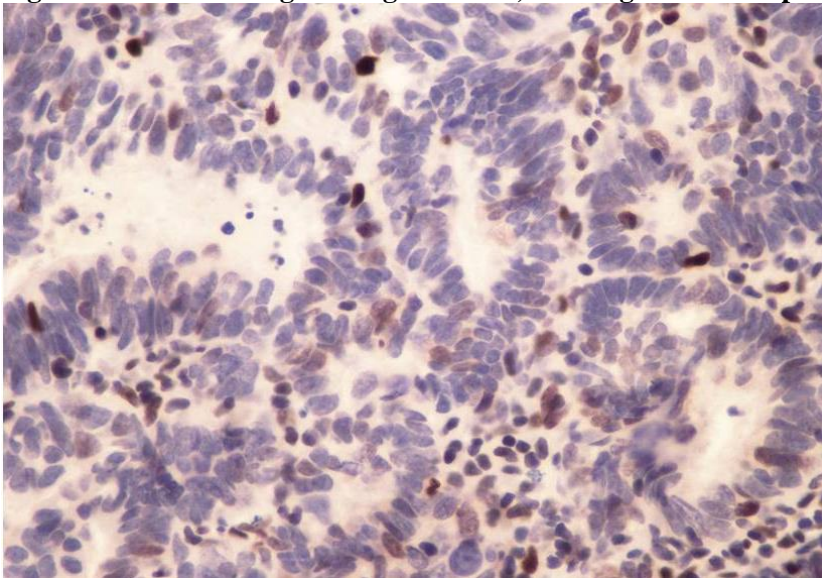
**MSH6: MSH6 weak staining can be seen with MSH2 loss.** We have observed this in 2 cases (one each from Vancouver General Hospital and Lion's Gate Hospital; both were endometrial carcinomas, and these were from a total of more than 3000 cases assessed, most with IHC for all four MMR proteins, and we have included photomicrographs from these two cases in *Supplementary Figures 1 to 3*), but it became very clear in this run that this is far from an isolated occurrence. It is likely that the phenomenon is exaggerated by the small cores of tissue used in the tissue microarray, **but please take into account the possibility that weak MSH6 expression may be associated with absence of expression of MSH2**, especially if you are using two markers (PMS2 and MSH6) for assessment of MMR status. We don't know if any of these patients with absent MSH2 expression and weak/focal MSH6 expression have Lynch Syndrome and this is clearly an area where further study is needed. In the meantime, as the goal is to screen for potential Lynch Syndrome cases, it would be prudent to refer these patients on to hereditary cancer program for further testing. Note that there was also heterogeneity of MSH6 expression in core 16, which was considered to be a case with absent MSH6 expression when the cases were selected for this run. This suggests that this case is likely to be one with epigenetic silencing of MSH6 rather than a germline mutation, but we unfortunately do not know what the germline status of MSH6 is in this case. **SUMMARY: If we exclude the cases with MSH2 loss, were there was variable MSH6 expression, as noted previously, and core 16 which also showed heterogeneity of MSH6 staining, then the results were completely concordant, except for one lab which recorded absent staining for MSH6 in core 33 (Lab 189, slide not available for review).**

Garrattograms after cIQC assessment of MLH1, PMS2, MSH2 and MSH6 are provided in Supplementary Figures 4 to 7. 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.

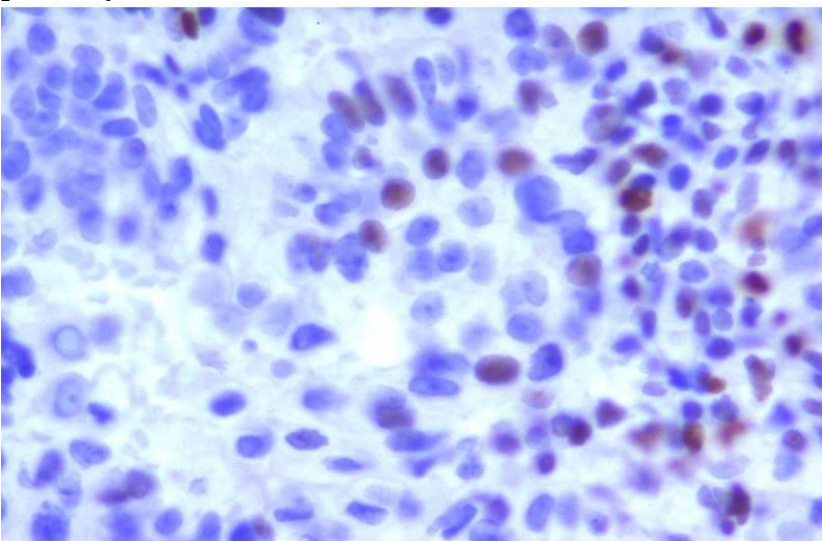
**Figure S1. Case 1 – Endometrial carcinoma with weak focal expression of MSH6. Note strong expression in adjacent benign glands (lower right). MSH2 was absent/deleted.**



**Figure S2. Case 1 – Higher magnification, showing weak focal positivity for MSH6 within tumour cell nuclei.**



**Figure S3. Case 2 – Endometrial carcinoma with weak focal MSH6 expression (a few tumour cell nuclei staining positively). MSH2 was absent/deleted.**









**Figure S7. Garrattogram after cIQc assessment of MSH6 IHC.**

Lab/ Core	101	102	106	107	109	110	111	112	113	114	115	116	124	125	138	141	144	145	149	160	164	170	175	181	184	186	189	191	202	207	210	215	217	220	221	R1		
1	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	E	E	PMS2	
2	U	A	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	U	A	U	A	A	A	A	A	E	A	A	F	A	A	A	A	A	A	A	A	MSH2
3	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	NORMAL	
4	A	A	A	A	A	A	A	A	A	A	A	E	A	E	A	A	A	E	A	A	A	E	A	A	E	F	A	U	A	A	A	A	A	A	A	A	A	MSH2
5	U	A	A	U	A	A	U	U	A	U	A	A	U	A	E	A	A	F	A	U	F	A	A	A	A	A	F	A	A	U	A	A	A	U	U	U	MSH6	
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	E	E	NORMAL	
7	E	U	U	E	U	E	E	U	E	E	E	E	F	E	E	E	E	E	E	U	E	E	U	U	U	E	U	U	U	U	F	U	U	U	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	F	A	A	A	A	F	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	E	E	MLH1	
10	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	F	E	E	E	E	E	E	E	E	E	MLH1	
11	E	E	E	E	E	E	E	E	E	E	E	E	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	A	U	U	U	U	U	U	A	A	U	U	U	U	U	U	U	A	A	U	A	U	A	U	A	U	A	A	A	U	A	F	U	U	A	A	MSH2		
13	E	E	E	E	E	U	E	E	E	U	E	E	U	U	E	E	E	E	U	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	NORMAL	
14	U	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	F	E	U	E	E	NORMAL		
15	E	E	E	E	E	E	E	E	E	E	E	E	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	
16	A	A	A	E	A	A	A	F	A	A	A	A	A	A	A	F	A	A	A	A	E	E	A	A	E	A	F	E	A	A	F	E	E	A	F	MSH6		
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	E	NORMAL	
18	A	E	A	A	A	A	A	A	E	A	A	A	A	A	E	A	A	A	A	A	A	A	A	A	E	A	A	A	E	E	A	F	E	E	F	A	MSH2	
19	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	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	E	E	E	E	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	NORMAL	
22	A	E	E	A	A	A	E	A	E	A	E	A	A	A	E	A	F	E	A	A	A	A	A	A	A	E	A	A	A	A	E	A	A	E	A	A	MSH2	
23	E	E	E	E	E	E	E	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	U	MLH1	
24	A	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	F	A	U	A	U	U	U	A	A	MSH6		
25	E	E	E	E	E	E	U	U	E	E	E	E	E	E	E	U	U	E	U	E	E	E	U	E	E	E	E	E	E	U	E	E	E	E	E	E	NORMAL	
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	E	E	E	E	E	NORMAL	
27	E	E	E	E	E	E	E	U	U	E	E	U	E	E	E	U	E	E	U	E	E	E	E	E	E	E	E	F	E	E	E	E	E	E	E	E	PMS2	
28	E	E	E	E	E	E	E	E	E	E	E	E	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	
29	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	U	E	E	E	E	E	E	NORMAL	
30	E	E	E	E	E	E	E	E	E	E	E	E	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	
31	E	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	F	U	U	A	F	MSH6		
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	E	E	E	E	E	NORMAL	
33	E	E	E	F	E	E	E	F	E	E	F	E	F	E	E	F	E	E	F	E	E	F	E	E	E	E	E	A	E	E	E	F	E	E	F	E	NORMAL	
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	A	A	A	A	F	A	A	A	A	A	A	MSH2
35	E	E	E	E	E	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	
36	E	E	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	E	E	E	E	E	E	E	E	E	PMS2
37	E	E	E	U	U	U	E	E	U	U	U	U	U	U	U	U	E	E	U	E	U	E	U	E	U	E	U	E	E	U	E	E	E	E	E	E	NORMAL	
38	A	E	A	A	A	A	A	A	A	A	E	A	A	A	A	F	E	A	A	A	A	A	A	A	A	F	A	F	E	A	A	A	U	A	A	A	MSH2	
39	E	E	E	E	E	E	E	E	E	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	MLH1	
40	E	E	E	E	E	E	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	NORMAL	

**Table S1. Reported MLH1 staining protocols.**

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time (mins)	Antibody Clone	Antibody Dilution	Antibody Supplier/Vendor	Antibody Lot #	Ab Incubation Time (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	64 minutes	ES05	1:100	Leica	6023826	32 minutes	OptiView	N	Y	DAB
102	DAKO PT 3IN1 High pH	10/20/10	ES05	1:80	DAKO	10086379	30" RT	DAKO FLEX	NO	CUSO4	DAB+
106	microwave pressure cooker	30 minutes	ES05	1:80	Novocastra	3025203	45 minutes	MACH4	no	no	DAB
107	CCI	64	ES05	1:20	Novocastra	6023826	32	Optiview DAB	N	Y	DAB
109	HIER high pH (CC1)	32 MIN	M1	RTU	VENTANA	E03021	16 MIN	OPTIVIEW	NO	YES	DAB
110	PT TANK PH9	20 @ 97C	ES05	1:50	DAKO	10094898	20	Dako Flex HRP	Y	N	DAB
111	CC1	64 MIN	G168-15	1/20	BD PHARMINGEN	3256964	32 MIN	ULTRAVIEW	YES	NO	DAB/COPPER
112	BOND Epitope Retrieval 2	25 minutes	ES05	1:100	Leica (Novocastra)	6023826	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	30'	ES05	1/90	DAKO	10099150	20'	Flex +	Y	Y	DAB
114	CC1	32	ES05	1/50	Leica	6023826	44	Optiview	n	y	DAB
115	EnVFlex Trs high PH	30	ES05	prediluted	Dako	10091391	20	envisionflex	N	N	enV Flex substrate solution 5 min
116	CC1	56 min	G168-15	1/80	BD Pharmingen	2138711	44 min	Optiview DAB IHC	Yes	.	DAB
124	CC1	64 min.	ES05	1/100	Leica	6025230	16	Opti View	n	n	Dab
125	ER2-20	20	ES05	1/100	DAKO	10092577	15	Bond Polymer Refine	n	y	DAB
138	HIER pH9	20	ES05	RTU	Dako	10099150	20	Polymer	Y	N	DAB
141	HIER	20 mins	ES05	1:50	Dako	10094898	20 min	Polymer	Y	N	DAB
144	CC1	56 min.	ES05	1:25	Leica	6025230	32 min.	Optiview	N	Y Copper	DAB
145	CC1	56	G168-15	1/50	BIOCARE	20215	32	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	HIER PT LINK	30 min high pH	ES05	RTU	DAKO	10096234	30 min	EnVision Flex	No	No	DAB
160	CC1 100 CELSIUS	76 MIN	M1	PRE-DILUTED	VENTANA	F01124	24 MIN	ULTRAVIEW	N	COPPER CUSO4	DAB
164	ultraCC1	16	M1	predilute	Ventana	E10276	32	OptiView	N	N	DAB
170	Hier ph 9.0	20 min	ES05	ready to use	Dako	10095907	20 min	Envision Flex Dako	N	N	DAB
175	Heat - CC1	64 min	M1	pre-dilute	Roche	E10276 exp 12/16	16 ,om	Opti-view	N	Y-copper	DAB
181	HIER	20 min	ES05	1:50	Dako	10094898	20 min	Polymer	Y	N	DAB
184	HIER (PT-Link, High pH)	20 min	ES05	RTU	Dako	10099150	20 min	FLEX+	Y	N	DAB
186	HEIR	20	G168-728	1:50	Cell Marque	1315706A	15	Leica Bond Plymer	N	N	DAB
189	CC1	64	M1	Pre-dilute	Ventana	NA	16	Ultraview	N	N	DAB
191	CC1	60'	ES05	1/10	novocastra	6025230	16'	ultraview DAB	Y	N	DAB
202	er2	20 min	es05	1/10	BD/Pharmagen	5065531	15 min	refine detection kit	no	no	dab
207	Heat Retrieval-Borg	118Å°C for 2 min	G168-15	1/200	Biocare	20215	60min	Mach 3	N	Y	DAB
210	N/A	N/A	E505	RTU	DAKO	N/A	30	Envision flex+	Mouse linker	N/A	N/A
215	CC1	64	G168-15	1:30	Biocare Medical	71715	60 mins	Optiview	Y	N	DAB
217	HIER	64	M1	predilute	Ventana	790-4535	60	optiview	N	Y	DAB
220		72	ES05		Dako		60	Opti-View	N	N	
221	PTM - pH9 Tris/EDTA	20	ES05	1:50, Renoir Diluent	Biocare	20215	60	Mouse EnVision	N	N	DAB



**Table S2. Reported PMS2 staining protocols.**

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time (mins)	Antibody Clone	Antibody Dilution	Antibody Supplier/Vendor	Antibody Lot #	Ab Incubation Time (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	EP51	1:15	Epitomics	EM022410	32 minutes	Optiview	N	COPPER	DAB
102	DAKO PT 3in1 High pH	10/20/10	EP51	1:25	DAKO	10010035	30" RT	DAKO FLEX	NO	CUSO4	DAB+
106	microwave pressure cooker	30 min	MRQ-28	1:15	Cell Marque	1430403-B	45 minutes	MACH4	no	no	DAB
107	Decloak Flex TRS High pH 9.0	30 minutes	A16-4	1:200	BD Pharmigen	5051647	30 minutes	Flex + 30	N	N	DAB
109	ultra CC1 (High pH)	64 min	EPR3947	RTU	CELL MARQUE	1329701D	20 MIN	OPTVIEW	YES	yes	DAB
110	PT TANK PH9	20 MIN @ 97C	EP51	1:50	DAKO	10095536	30 MIN	Dako Flex HRP	N	N	DAB
111	CC1	36 MIN	EPR3947	PREDILUTE	VENTANA	1417001E	32 MIN	ULTRAVIEW	YES	NO	DAB/COPPER
112	BOND Epitope Retrieval 2	30 minutes	EP51	1:75 using Background reducing diluent	DAKO	10100352	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	30'	PMS2	1/20	BD	5174534	30'	Flex +	Y	N	DAB
114	CC1	64	EP51	1/25	Epitomics	EL090506	64	Optiview	n	y	DAB
115	EnVFlex Trs high PH	30	EP51	prediluted	Dako	10092835	20	envisionflex	N	N	enV Flex substrate solution 5 min
116	CC1	64 min	EPR3947	RTU	Ventana Roche	1417001E	60 min	Optiview DAB IHC	Y	.	DAB
124	CC1	32 min.	EP51	1/40	Dako	10096211	32	Opti View	n	n	Dab
125	ER2-20	20	A16-4	1/75	BD Biosciences	4154997	15	Bond Polymer Refine	n	y	DAB
138	HIER pH9	20	EP51	RTU	Dako	10096479	30	Polymer	N	N	DAB
141	HIER	20 mins	EP51	1:50	Dako	10095536	30 mins	Polymer	N	N	DAB
144	CC1	64 min	EPR3947	Pre-Dilute	Cell Marque	1506813B	60 min	Optiview	N	Y Copper	DAB
145	CC1	56	A16-4	1/45	BIOCARE	92214	28	VENTANA XT OPTVIEW ihc v4	n	n	DAB
149	HIER PT Link high pH	20 min 97 C	EP51	RTU	DAKO	10096479	20 min	EnVision Flex	Yes	No	DAB
160	CC1 100 CELSIUS	92 MIN	EPR3947	PRE-DILUTED	VENTANA	1417001E	2H	ULTRAVIEW	Y	COPPER CUSO4	DAB
164	ultraCC1	56	EPR3947	predilute	Ventana	1417001C	32	OptiView	N	N	DAB
170	Hier ph 9.0	20 min	EP51	ready to use	Dako	10100023	20 min	Envision Flex Dako	N	N	DAB
175	Heat - CC1	64 minutes	EPR3947	pre-dilute	Roche	1506813B exp 2-18	32 min	Opti-view	Y	Y-copper	DAB
181	HIER	20 min.	EP51	1:50	Dako	10095536	30 min	Polymer	N	N	DAB
184	HIER (PT-Link, High pH)	20 min	EP51	RTU	Dako	10096479	30	FLEX	N	N	DAB
186	HEIR	20	EPR3947	1:2	Cell Marque	1417001J	15	Leica Bond Polymer	N	N	DAB
189	CC1	64	EPR3947	Pre-diute	Ventana/Cell Marque	NA	32	Ultraview	Y	N	DAB
191	CC1	64'	ERP3947	RTU	Roche	1417001e	48'	optiview DAB	12'	N	DAB
202	BOND ER 2	30 MIN	A16-4	1/25	Becton Dickinson/pharmagen	5174534	15 min	refine detection kit	no	no	dab
207	Heat Retrieval-citrate	118Å°C for 2 min	A16-4	1/100	BD	5051647	60 min	Mach 3	N	Y	DAB
210	High	N/A	EP51	RTU	DAKO	N/A	30	Envision flex+	N/A	Mouse linker	N/A
215	CC1	64 mins	EPR3947	Predilute	Ventana	1417001E	40min	Optiview	Y	N	DAB
217	HIER	92 min	EPR3947	predilute	Ventana	760-4531	96 min	optiview	N	Y	DAB
220		64min	EPR3947				72min	Opti-View	N	N	
221	PTM - pH9 Tris/EDTA	20	EP51	1:25, Renoir Diluent	Biocare	10815	60	Rabbit EnVision	N	N	DAB

**Table S3. Reported MSH2 staining protocols.**

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time (mins)	Antibody Clone	Antibody Dilution	Antibody Supplier/Vendor	Antibody Lot #	Ab Incubation Time (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	G219-1129	1:200	Cell Marque	1313003B	32 minutes	Optiview	N	COPPER	DAB
102	DAKO PT 3in1 High pH	10/20/10	FE11	1:50	DAKO	10080535	30* RT	DAKO FLEX	NO	CUS04	DAB+
106	microwave pressure cooker	30 minutes	25D12	1:25	Novocastra	6030020	45 minutes	MACH4	no	no	DAB
107	CCI	32	G219-1129	1:200	Cell Marque	1334002A	32	Optiview DAB	N	Y	DAB
109	HIER CC1-HIGH pH	32 MIN	G219-1129	RTU	CELL MARQUE	1334003C	8 MIN	OPTIVIEW	NO	YES	DAB
110	PT TANK PH9	20 @ 97C	FE11	1:150	DAKO	10096498	20 min	Dako Flex HRP	Y	N	DAB
111	CC1	64 MIN	G219-1129	1\400	BD PHARMINGEN	2258829	32 MIN	iVIEW	NO	BLOCKER	DAB/COPPER
112	BOND Epitope Retrieval 2	30 minutes	FE11	1:100	DAKO	10098628	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	30'	25D12	1/25	LEICA/Novocastra	6035858	30'	Flex +	Y	N	Y (DAB)
114	CC1	32	G219-1129	1/100	Cell Marque	1313003F	32	Optiview	n	y	DAB
115	EnVFlex Trs high PH	30	FE11	prediluted	Dako	1092042	20	envisionflex	N	N	enV flex substrate solution 5min
116	CC1	48 min	G219-1129	1/400	Cell Marque	1313003 B	48 min	Optiview DAB IHC	NO	.	DAB
124	CC1	24 min.	G219-1129	prediluted	Cell Marque	1417104F	16 min.	Opti View	n	n	Dab
125	ER2-20	20	25D12	1/100	Novocastra	6035858	15	Bond Polymer Refine	n	y	DAB
138	HIER pH9	20	FE11	RTU	Dako	10099381	20	Polymer	Y	N	DAB
141	HIER	20 mins	FE11	1:150	Dako	10096498	20 mins	Polymer	Y	N	DAB
144	CC1	40 min	G219-1129	Pre-Dilute	Cell Marque	1417104D	16 min.	Optiview	N	Y Copper	DAB
145	CC1	40	G219-1129	1/400	CELLMARQUE	1313003A	24	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	HIER PT LINK high pH	20 min 97 C	FE11	RTU	DAKO	10096663	20 min	EnVision Flex	Yes	No	DAB
160	CC1 100 CELSIUS	64 MIN	G219-1129	PRE-DILUTED	VENTANA	1417104F	48 MIN	ULTRAVIEW	N	COPPER CUS04	DAB
164	ultraCC1	16	G219-1129	predilute	Ventana	1417104D	16	OptiView	N	N	DAB
170	Hier ph 9.0	20 min	FE11	ready to use	Dako	10095518	20 min	Envision Flex Dako	N	N	DAB
175	Heat - CC1	32 min	G219-1129	pre-dilute	Roche	1417104F exp 5/17	16 min	Opti-view	N	Y-copper	DAB
181	HIER	20 min	FE11	1:150	Dako	10096498	20 min	polymer	Y	n	DAB
184	HEIR (PT-Link, High pH)	20 min	FE11	RTU	Dako	10092042	20 min	FLEX+	Y	N	DAB
186	HIER	20	G219-1129	1:200	Cell Marque	1313003A	15	Leica Bond Polymer	N	N	DAB
189	CC1	32	G219-1129	Pre-dilute	Ventana/Cell Marque	NA	16	Ultraview	N	N	DAB
191	CC1	32'	G219-1129	RTU	Roche	1417104E	16'	optiview DAB	N	N	DAB
202	er2	30 min	25d12	rtu	leica	30048	15 min	refine detection kit	n	n	dab
207	Heat Retrieval-Borg	118Å°C for 2 min	FE11	1/4000	CALBIOCHEM	86848	60min	Mach 3	N	Y	DAB
210	N/A	N/A	FE11	RTU	Dako	N/A	20	Envision flex+	N/A	Mouse linker	N/A
215	CC1	24	E219-1129	Predilute	Cell Marque	1417104L	16	Ultraview	N	N	DAB
217	HIER	56 min	G2191129	predilute	Ventana	7604265	32	optiview	N	Y	DAB
220		32	G219-1129		Cell Marque		32	Opti-View	No	No	
221	PTM - pH9 Tris/EDTA	20	FE11	1:25, Renoit Diluent	Dako	92214	60	Mouse EnVision	N	N	DAB

**Table S4. Reported MSH6 staining protocols.**

Lab ID	Antigen Retrieval Method	Antigen Retrieval Time (mins)	Antibody Clone	Antibody Dilution	Antibody Supplier/Vendor	Antibody Lot #	Ab Incubation Time (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	EP49	1:200	Epitomics	EL120401	32 minutes	OptiView	N	Y	DAB
102	DAKO PT 3in1 High pH	10/20/10	EP49	1:100	DAKO	10079041	30" RT	DAKO FLEX	NO	CUSO4	DAB+
106	microwave pressure cooker	30 minutes	SP93	1:15	Cell Marque	1512802B	45 minutes	MACH4	no	no	DAB
107	CCI	60	44/MSH6	1:300	BD	4339757	32	Ultraview DAB	N	Y	DAB
109	HIER high pH (CC1)	64 MIN	44	RTU	VENTANA	E02714	20 MIN	OPTIVIEW	NO	YES	DAB
110	PT TANK PH9	20 @ 97C	EP49	1:300	DAKO	10096686	30 MIN	Dako Flex HRP	N	N	DAB
111	CC1	64 MIN	BC/44	PREDILUTE	BIOCARE MEDICAL	111214	32 MIN	ULTRAVIEW	NO	NO	DAB/ COPPER
112	BOND Epitope Retrieval 2	40 minutes	EP49	1:1500	Epitomics	EL-120401	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	30'	EP49	Predilute	DAKO	10099688	15'	Flex	Y	Y	DAB
114	CC1	64	EP49	1/200	Epitomics	CI090101	32	Optiview	n	y	DAB
115	EnVFlex Trs high PH	30	EP49	prediluted	Dako	10092289	20	envisionflex	N	N	enV Flex substrate solution 5 min
116	CC1	40 min	BC/44	1/400	Boicare Medical	70814	48 min	Optiview DAB IHC	Yes	.	DAB
124	CC1	32 min.	EP49	1/50	Dako	10100346	32 min.	Opti View	n	n	Dab
125	ER2-20	20	44/MSH6	1/1500	BD Biosciences	11917	15	Bond Polymer Refine	n	y	DAB
138	HIER pH9	20	EP49	RTU	Dako	10095909	RTU	Polymer	N	N	DAB
141	HIER	20 mins	EP49	1:300	Dako	10096686	30 mins	Polymer	N	N	DAB
144	CC1	32 min.	EP48	1:100	Cedarlane	eLO61801	32 min.	Optiview	N	Y Copper	DAB
145	CC1	32	44	1/300	CELLMARQUE	1313501A	16	VENTANA XT OPTIVIEW ihc v4	n	n	DAB
149	HIER PT LINK high pH	20 min 97 C	EP49	RTU	DAKO	10097795	20 min	EnVision Flex	No	No	DAB
160	CC1 100 CELSIUS	92 MIN	44	PRE- DILUTED	VENTANA	F01005	1H	ULTRAVIEW	N	COPPER CUSO4	DAB
164	ultraCC1	16	44	predilute	Ventana	E09695	32	OptiView	N	N	DAB
170	Hier pH9.0	20 min	EP49	ready to use	Dako	10095909	20 min	Envision Flex Dako	N	N	DAB
175	Heat - CC1	64 min	44	1/100	Cell Marque	1112208C	24 in	Opti-view	Y	Y-copper	DAB
181	HIER	20 min	EP49	1:300	Dako	10096686	30 min	polymer	N	N	DAB
184	HEIR (PT-Link, High pH)	20	EP49	RTU	Dako	10095022	20	FLEX	N	N	DAB
186	HEIR	20	BC/44	1:50	Biocare Medical	11714	15	Leica Bond Polymer	N	N	DAB
189	CC1	64	44	Pre-dilute	Ventana	NA	16	Ultraview	N	N	DAB
191	CC1	60'	EP49	1/25	Dako	10100348	60'	ultraview DAB	y	n	DAB
202	er2	40min	pu29	1/25	abcam	gr189185	15 min	refine detection kit	no	no	dab
207	Heat Retrieval-Borg	118Å°C for 2 min	44	1/400	BD	4050545	60 MIN	MACH 3	N	Y	DAB
210	High	N/A	EP49	RTU	Dako	N/A	20	Envision flex+	N/A	N/A	N/A
215	CC1	56	EPR3945	1:100	ABCAM	ER189185-12	32	Ultraview	N	N	DAB
217	HIER	64	GTBP-45	1:3000	Ventana	610919	60	optiview	N	Y	DAB
220		32	EP49				28	OptiView	N	N	
221	PTM - pH9 Tris/EDTA	20	EP49	1:50, Renoir Diluent	Dako	20215	60	Rabbit EnVision	N	N	DAB

**Table S5. Descriptive statistics for MLH1 based on cIQc assessment (cores 27 and 39 excluded).**

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	38	89.47	34	34/34 (100%)	1	1	1	1	1
102	38	86.84	33	33/33 (100%)	1	1	1	1	1
106	38	86.84	33	33/33 (100%)	1	1	1	1	1
107	38	86.84	33	33/33 (100%)	1	1	1	1	1
109	38	81.58	31	31/31 (100%)	1	1	1	1	1
110	38	86.84	33	33/33 (100%)	1	1	1	1	1
111	38	84.21	32	32/32 (100%)	1	1	1	1	1
112	38	86.84	33	33/33 (100%)	1	1	1	1	1
113	38	86.84	33	33/33 (100%)	1	1	1	1	1
114	38	92.11	35	35/35 (100%)	1	1	1	1	1
115	38	81.58	31	31/31 (100%)	1	1	1	1	1
116	38	89.47	34	34/34 (100%)	1	1	1	1	1
124	38	89.47	34	34/34 (100%)	1	1	1	1	1
125	38	89.47	34	34/34 (100%)	1	1	1	1	1
138	38	76.32	29	29/29 (100%)	1	1	1	1	1
141	38	89.47	34	34/34 (100%)	1	1	1	1	1
144	38	94.74	36	36/36 (100%)	1	1	1	1	1
145	38	94.74	36	36/36 (100%)	1	1	1	1	1
149	38	92.11	35	35/35 (100%)	1	1	1	1	1
160	38	86.84	33	33/33 (100%)	1	1	1	1	1
164	38	86.84	33	33/33 (100%)	1	1	1	1	1
170	38	94.74	36	36/36 (100%)	1	1	1	1	1
175	38	89.47	34	34/34 (100%)	1	1	1	1	1
181	38	89.47	34	34/34 (100%)	1	1	1	1	1
184	38	86.84	33	33/33 (100%)	1	1	1	1	1
186	38	94.74	36	36/36 (100%)	1	1	1	1	1
189	38	92.11	35	35/35 (100%)	1	1	1	1	1
191	38	94.74	36	36/36 (100%)	1	1	1	1	1
202	38	76.32	29	29/29 (100%)	1	1	1	1	1
207	38	84.21	32	32/32 (100%)	1	1	1	1	1
210	38	92.11	35	35/35 (100%)	1	1	1	1	1
215	38	94.74	36	36/36 (100%)	1	1	1	1	1
217	38	81.58	31	31/31 (100%)	1	1	1	1	1
220	38	92.11	35	35/35 (100%)	1	1	1	1	1
221	38	97.37	37	37/37 (100%)	1	1	1	1	1

**Table S6. Descriptive statistics for PMS2 based on cIQc assessment (cores 27 and 39 excluded).**

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	38	89.47	34	34/34 (100%)	1	1	1	1	1
102	38	76.32	29	29/29 (100%)	1	1	1	1	1
106	38	84.21	32	32/32 (100%)	1	1	1	1	1
107	38	97.37	37	37/37 (100%)	1	1	1	1	1
109	38	81.58	31	31/31 (100%)	1	1	1	1	1
110	38	89.47	34	34/34 (100%)	1	1	1	1	1
111	38	89.47	34	34/34 (100%)	1	1	1	1	1
112	38	89.47	34	34/34 (100%)	1	1	1	1	1
113	38	86.84	33	33/33 (100%)	1	1	1	1	1
114	38	92.11	35	35/35 (100%)	1	1	1	1	1
115	38	86.84	33	33/33 (100%)	1	1	1	1	1
116	38	84.21	32	32/32 (100%)	1	1	1	1	1
124	38	89.47	34	34/34 (100%)	1	1	1	1	1
125	38	84.21	32	32/32 (100%)	1	1	1	1	1
138	38	81.58	31	31/31 (100%)	1	1	1	1	1
141	38	81.58	31	31/31 (100%)	1	1	1	1	1
144	38	92.11	35	35/35 (100%)	1	1	1	1	1
145	38	89.47	34	34/34 (100%)	1	1	1	1	1
149	38	92.11	35	35/35 (100%)	1	1	1	1	1
160	38	86.84	33	33/33 (100%)	1	1	1	1	1
164	38	86.84	33	33/33 (100%)	1	1	1	1	1
170	38	94.74	36	36/36 (100%)	1	1	1	1	1
175	38	89.47	34	34/34 (100%)	1	1	1	1	1
181	38	92.11	35	35/35 (100%)	1	1	1	1	1
184	38	86.84	33	33/33 (100%)	1	1	1	1	1
186	38	92.11	35	35/35 (100%)	1	1	1	1	1
189	38	92.11	35	35/35 (100%)	1	1	1	1	1
191	38	92.11	35	35/35 (100%)	1	1	1	1	1
202	38	76.32	29	29/29 (100%)	1	1	1	1	1
207	38	78.95	30	30/30 (100%)	1	1	1	1	1
210	38	78.95	30	30/30 (100%)	1	1	1	1	1
215	38	92.11	35	35/35 (100%)	1	1	1	1	1
217	38	84.21	32	32/32 (100%)	1	1	1	1	1
220	38	89.47	34	34/34 (100%)	1	1	1	1	1
221	38	89.47	34	34/34 (100%)	1	1	1	1	1

**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	40	90	36	36/36 (100%)	1	1	1	1	1
102	40	82.5	33	33/33 (100%)	1	1	1	1	1
106	40	85	34	34/34 (100%)	1	1	1	1	1
107	40	87.5	35	35/35 (100%)	1	1	1	1	1
109	40	90	36	36/36 (100%)	1	1	1	1	1
110	40	90	36	36/36 (100%)	1	1	1	1	1
111	40	90	36	36/36 (100%)	1	1	1	1	1
112	40	82.5	33	33/33 (100%)	1	1	1	1	1
113	40	90	36	36/36 (100%)	1	1	1	1	1
114	40	87.5	35	35/35 (100%)	1	1	1	1	1
115	40	82.5	33	33/33 (100%)	1	1	1	1	1
116	40	90	36	36/36 (100%)	1	1	1	1	1
124	40	85	34	34/34 (100%)	1	1	1	1	1
125	40	92.5	37	37/37 (100%)	1	1	1	1	1
138	40	90	36	36/36 (100%)	1	1	1	1	1
141	40	90	36	36/36 (100%)	1	1	1	1	1
144	40	90	36	36/36 (100%)	1	1	1	1	1
145	40	92.5	37	37/37 (100%)	1	1	1	1	1
149	40	90	36	36/36 (100%)	1	1	1	1	1
160	40	77.5	31	31/31 (100%)	1	1	1	1	1
164	40	90	36	36/36 (100%)	1	1	1	1	1
170	40	90	36	36/36 (100%)	1	1	1	1	1
175	40	90	36	36/36 (100%)	1	1	1	1	1
181	40	95	38	38/38 (100%)	1	1	1	1	1
184	40	87.5	35	35/35 (100%)	1	1	1	1	1
186	40	95	38	38/38 (100%)	1	1	1	1	1
189	40	92.5	37	37/37 (100%)	1	1	1	1	1
191	40	90	36	36/36 (100%)	1	1	1	1	1
202	40	82.5	33	33/33 (100%)	1	1	1	1	1
207	40	85	34	34/34 (100%)	1	1	1	1	1
210	40	90	36	36/36 (100%)	1	1	1	1	1
215	40	87.5	35	35/35 (100%)	1	1	1	1	1
217	40	90	36	36/36 (100%)	1	1	1	1	1
220	40	95	38	38/38 (100%)	1	1	1	1	1
221	40	95	38	38/38 (100%)	1	1	1	1	1

**Table S8. Descriptive statistics for MSH6 based on cIQc assessment (cores 2, 4, 12, 16, 18, 22 and 38 excluded).**

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	33	87.88	29	28/29 (97%)	1	0.75	0.96	1	0.84
102	33	87.88	29	29/29 (100%)	1	1	1	1	1
106	33	84.85	28	28/28 (100%)	1	1	1	1	1
107	33	87.88	29	29/29 (100%)	1	1	1	1	1
109	33	87.88	29	29/29 (100%)	1	1	1	1	1
110	33	87.88	29	29/29 (100%)	1	1	1	1	1
111	33	87.88	29	29/29 (100%)	1	1	1	1	1
112	33	84.85	28	28/28 (100%)	1	1	1	1	1
113	33	81.82	27	27/27 (100%)	1	1	1	1	1
114	33	81.82	27	27/27 (100%)	1	1	1	1	1
115	33	90.91	30	30/30 (100%)	1	1	1	1	1
116	33	90.91	30	30/30 (100%)	1	1	1	1	1
124	33	81.82	27	27/27 (100%)	1	1	1	1	1
125	33	87.88	29	29/29 (100%)	1	1	1	1	1
138	33	90.91	30	29/30 (97%)	1	0.67	0.96	1	0.78
141	33	90.91	30	30/30 (100%)	1	1	1	1	1
144	33	84.85	28	28/28 (100%)	1	1	1	1	1
145	33	90.91	30	30/30 (100%)	1	1	1	1	1
149	33	87.88	29	29/29 (100%)	1	1	1	1	1
160	33	75.76	25	25/25 (100%)	1	1	1	1	1
164	33	90.91	30	30/30 (100%)	1	1	1	1	1
170	33	93.94	31	31/31 (100%)	1	1	1	1	1
175	33	84.85	28	28/28 (100%)	1	1	1	1	1
181	33	87.88	29	29/29 (100%)	1	1	1	1	1
184	33	87.88	29	29/29 (100%)	1	1	1	1	1
186	33	93.94	31	31/31 (100%)	1	1	1	1	1
189	33	90.91	30	29/30 (97%)	0.96	1	1	0.8	0.87
191	33	93.94	31	31/31 (100%)	1	1	1	1	1
202	33	84.85	28	28/28 (100%)	1	1	1	1	1
207	33	81.82	27	27/27 (100%)	1	1	1	1	1
210	33	93.94	31	31/31 (100%)	1	1	1	1	1
215	33	90.91	30	30/30 (100%)	1	1	1	1	1
217	33	84.85	28	28/28 (100%)	1	1	1	1	1
220	33	93.94	31	31/31 (100%)	1	1	1	1	1
221	33	93.94	31	31/31 (100%)	1	1	1	1	1