

Assessors' report for cIQc Run 46: MMR Immunohistochemistry

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Assessment performed on Monday, May 4th 2015 at St. Boniface Hospital, Winnipeg.

Overview

A total of 36 labs participated in Run 46, which is 15 more labs compared to the previous MMR challenge (Run 38) in 2014. Only Lab 200 did not return stained slides 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.

It was noted by assessors that an increased rate of “failed” tests is an indication of a technical lack of sensitivity of the test. While interpretation is correct in all cores that are interpretable, the screening for Lynch syndrome potentially becomes less accurate due to this poor sensitivity and increased likelihood of uninterpretable staining. A frequency graph of the number of failed cores in each participating laboratory is provided in Figure 1.

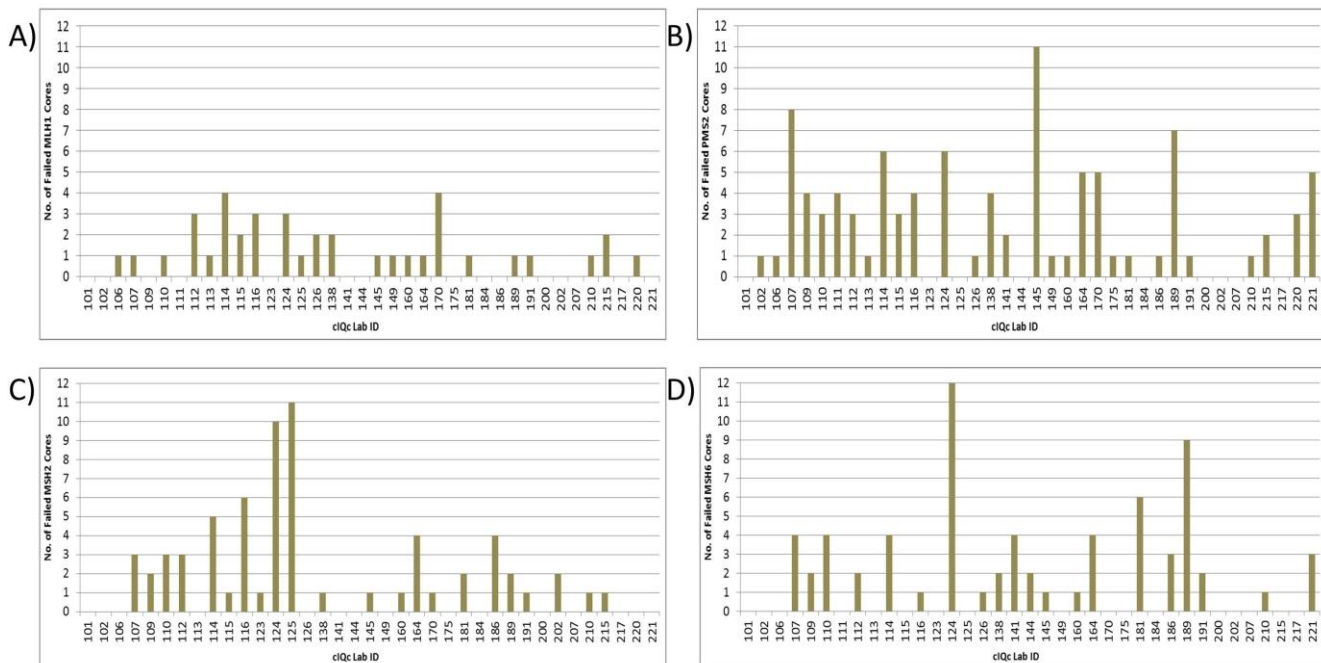


Figure 1. Frequency of failed cores in each participating laboratory. A) MLH1. B) PMS2. C) MSH2. D) MSH6.

An overall general improvement of staining from the 2014 challenge was observed, but a number of laboratories still performed poorly in the current MMR challenge. Select laboratories had particularly nice staining and we encourage all labs to look at the protocols used by these facilities mentioned specifically in the comments sections of this summary report.

Laboratories deemed by assessors to have *Failed* or *Sub-Optimal* staining should carefully review their protocols and re-calibrate staining. We understand that many laboratories are locked into detection systems, making it difficult to use protocols used by better performing laboratories verbatim. However, it should be possible to find a site using the same detection system that is achieving *Optimal* or *Adequate* scores. Please note that cIQc can provide material for calibration and validation.

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: Necrotic material and sampling issues made Core 22 difficult to interpret therefore assessors did not refer to this core. Assessors also noted and confirmed sampling variability in Core 4 across participants. Labs 106, 123 and 141 using the ES05 clone had Optimal staining using ES05. A single laboratory failed the assessment; and it was noted that this was the only lab using the epr3894 clone. Only Lab 112 achieved optimal staining with clone G168-15 while G168-728 was a poor performer with generalized weak staining.

Lab	IHC Status*	cIQc Comments
101	Sub-Optimal	Stromal staining weak in some cores. Poor internal controls. Increase in background staining. Some non-specific staining observed.
102	Optimal	Stromal staining weak in some cores
106	Optimal	Excellent staining
107	Optimal	Core 4 is confirmed to be expressing the MLH1 protein.
109	Adequate	Core 4 is confirmed to be expressing the MLH1 protein. Some weak stromal staining. This lab may consider increasing their antibody incubation time.
110	Optimal	
111	Adequate	Core 4 is confirmed to be expressing the protein. Stromal staining weak in some cores.
112	Optimal	Core 18 was considered unsatisfactory (U) for evaluation because it is mostly necrosis.
113	Optimal	Good quality staining
114	Sub-optimal	Generally very weak staining resulting in an increase in failed scores. This lab may consider increasing their antibody incubation time.
115	Sub-optimal	Very weak stromal staining; Generally poor internal controls
116	Adequate	Acceptable staining

123	Optimal	Excellent staining
124	Failed	Staining requires optimizing. Advise that the lab review the protocols of other laboratories using the same detection system. (i.e. Lab 107).
125	Adequate	General weak staining
126	Optimal	Good general staining
138	Optimal	Good quality staining
141	Optimal	Good quality staining
144	Sub-optimal	Increase of background staining. Requires further optimization. Short antigen retrieval time with a long Ab incubation time noted.
145	Sub-optimal	Internal controls lacking; General weak positivity and increased background
149	Optimal	Good staining
160	Optimal	Good staining
164	Sub-optimal	Assessors are concerned about the considerable background/cytoplasmic staining making the slide difficult to interpret. A short antigen retrieval time was noted.
170	Optimal	Cores 6 and 15 could be considered absent of expression, but understandable to also interpret as "Failed".
175	Adequate	Increased background staining, otherwise interpretable.
181	Optimal	Good staining
184	Adequate	Acceptable staining
186	Adequate	Lighter overall staining but adequate
189	Adequate	Generally weak staining throughout all the cores. Recommend the lab review the protocol of Lab 160 which had optimal results using the same system.
191	Optimal	Good quality, intense staining. Could possibly use this antibody at 1:20 dilution.
202	Sub-optimal	Increase background giving false positive staining. Recommend the lab review the protocol of Lab 112 that uses the same detection system.
207	Adequate	Good internal controls but general weak staining
210	Optimal	
215	Sub-optimal	Very weak staining resulting in increased number of failed cores.
217	Adequate	General background staining
220	Adequate	General background staining
221	Sub-optimal	Consistent lack of internal controls. A number of laboratories successfully use the same clone with Dako detection systems. Suggest reviewing protocols of other laboratories.

*Based on cIQc assessor consensus

PMS2: In the previous cIQc MMR (PMS2) challenge no “optimal” staining was observed. 50% of labs achieved optimal staining results in the current run, providing a demonstration that labs are improving their protocols with experience in using this antibody. There was a large variation in Ab incubation times noted, from 16 minutes to 2 hours. Sites using A16-4 seemed to have excessive background, though site 207 used A16-4 using mach-4 quite successfully. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Weak internal controls
102	Optimal	Good
106	Optimal	Excellent staining
107	Optimal	
109	Optimal	A little blush
110	Optimal	Good general staining
111	Sub-optimal	General weak staining
112	Adequate	Excessive background staining
113	Optimal	Good quality staining
114	Failed	Poor staining, very weak or non-existent. A large number of failed cores. It was noted that the antibody incubation time was very short at 16 minutes.
115	Sub-optimal	Excessive background
116	Optimal	RTU EPR3947 seems to working well
123	Adequate	Increased background but interpretable
124	Adequate	Very weak staining but interpretable
125	Adequate	Very weak staining but interpretable
126	Optimal	
138	Optimal	
141	Optimal	
144	Sub-optimal	Internal controls are generally weak to absent staining. Advise that this lab review protocols from other sites using the same detection system and antibody.
145	Failed	Excessive background, no stromal staining, not interpretable. An excessive number of failed cores.
149	Optimal	
160	Adequate	Generally weak but adequate staining. A 2-hour antibody incubation time was noted by the assessors.

164	Sub-optimal	Very weak staining. Advise that this lab review protocols form other sites using the same detection system and antibody.
170	Optimal	Good quality staining
175	Sub-optimal	Weak staining with increased background. Advise that this lab review protocols form other sites using the same detection system and antibody.
181	Optimal	
184	Adequate	Increase background and cytoplasmic staining
186	Optimal	
189	Sub-optimal	Weak staining throughout and difficult to interpret. Higher number of failed cores.
191	Optimal	Good internal controls
202	Sub-optimal	Increased background
207	Optimal	
210	Optimal	
215	Optimal	Good quality staining
217	Adequate	Some increase background staining
220	Adequate	Light staining overall but adequate for interpretation
221	Adequate	Slight increase in background staining. The high number of failed results was noted

*Based on cIQc assessor consensus

MSH2: Labs 106 and 141 provided excellent staining results. 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	Adequate	Very light staining. Poor internal controls
102	Optimal	Good staining
106	Optimal	Excellent staining
107	Optimal	
109	Optimal	
110	Optimal	Loss of some stromal cells staining
111	No slide	
112	Adequate	Light staining
113	Adequate	Very dark staining, overstained

114	Failed	Few or no internal controls present. High dilution of the antibody used and a very short incubation time was noted by the assessors.
115	Optimal	
116	Adequate	Weak staining but adequate for interpretation
123	Optimal	Good quality staining
124	Failed	Very weak staining with non-specific staining. A large number of failed cores noted.
125	Failed	Significant non-specific staining and generally weak
126	Optimal	Good quality staining
138	Optimal	Comment: Excellent strong specific staining
141	Optimal	Good quality consistent staining
144	Optimal	Lighter but very specific staining
145	Optimal	
149	Optimal	Excellent staining
160	Optimal	Some increased cytoplasmic blush
164	Adequate	Pale staining in some cores. A short stain protocol was noted.
170	Optimal	
175	Adequate	Increase background staining
181	Optimal	
184	Optimal	
186	Adequate	Overall weak staining but adequate
189	Sub-optimal	Strong endothelial cell staining. Weak and aberrant staining
191	Optimal	
202	Sub-optimal	Very light staining
207	Optimal	
210	Optimal	Expression in Core 29 was confirmed by assessors; possible sampling issue.
215	Optimal	
217	Adequate	Increased background staining
220	Optimal	Generally weak staining
221	Optimal	Slight background

MSH6: Clone 44 generally continued to show increased background staining that reduced specificity and sensitivity. With one exception, optimal staining results were achieved only by labs using the EP49 antibody. PU-29 was used by a single laboratory and it produced optimal results. Participant-specific feedback is summarized below:

Lab	IHC Status*	cIQc Comments
101	Adequate	Adequate. General weak staining
102	Adequate	Some over-staining
106	Optimal	High quality staining
107	Sub-optimal	Heavy background staining. A “muddy stain” difficult to interpret
109	Adequate	
110	Adequate	
111	Adequate	Increased background staining
112	Optimal	
113	Optimal	
114	Sub-optimal	Consistent weak staining throughout the cores
115	Adequate	Some precipitate
116	Adequate	Increase in background and cytoplasmic staining
123	Optimal	High quality staining
124	Sub-optimal	Extremely weak staining. Lack of internal controls in some cores
125	Adequate	
126	Optimal	Very nice stromal staining
138	Optimal	
141	Adequate	
144	Sub-optimal	Very weak staining
145	Adequate	Increased background; good stromal staining
149	Optimal	Good quality staining
160	Adequate	Weak staining but adequate
164	Adequate	Cytoplasmic background staining
170	Optimal	Good quality staining

175	Adequate	Increased (heavy) background staining
181	Adequate	
184	Optimal	
186	Sub-optimal	Weak or lacking internal controls
189	Failed	Very weak or completely lacking internal controls
191	Sub-optimal	High background staining. Overall weak picking up few internal controls
202	Optimal	Very nice staining
207	Adequate	Weak but adequate staining
210	Optimal	High quality staining
215	Adequate	Adequate towards optimal staining
217	Adequate	
220	Optimal	
221	Adequate	Some increase cytoplasmic and background staining

*Based on cIQc assessor consensus

Garrattograms after cIQc assessment of MLH1, PMS2, MSH2 and MSH6 are provided in Supplementary Figures 1 to 4. Supplementary Tables 1 to 4 summarizing staining protocols and Supplementary Tables 5 to 8 summarizing descriptive statistics can also be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Your regular participation in cIQc is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Table S1. Reported MLH1 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	ES05	1:20	Leica	6023826	32	OptiView	N	COPPER	DAB
102	Dako Flex High Target Retrieval Soln	10/20/10	ES05	1/80	DAKO	10077376	30' RT	DAKO FLEX+	NO	CUSO4	DAB+
106	TRIS HCl microwave pressure	30	ES05	1:75	Novacastra	6023826	45	MACH4	N	N	DAB
107	Ventana CCI	64	E505	1:20	NCL (Leica)	602 3826	32	Optiview DAB	N	Y (Copper)	DAB
109	ULTRA CC1 (high pH)at 100 degrees	32 MIN	M1	RTU	VENTANA	E03021	16 MIN	OPTIVIEW	NO	YES	DAB
110	heir	20 mins	ES05	1:50	DAKO	10086379	20 min	Polymer	Y	N	DAB
111	CC1	64 min	G168-15	1/20	BD Pharmingen	3256964	32 min	Ultraview	yes	Copper	DAB
112	Bond Epitope Retrieval 2	30 minutes	G168-15	1:100 Van Gogh Yellow diluent	BioCare Medical	120413	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	20min	ES05	Predilute	Dako	10096234	20min	Polymer	N	N	Dab
114	CC1	32 min	ES05	1:50	Leica	6023826	16	Optiview	N	Copper	DAB
115	Target retrieval enV flex Trs High pH, 97C	30min	ES05	prediluted	Dako	10091391	20min	envison flex	N	N	enV Flex substrate solution 5min
116	CC1	48 min	G168-15	1/80	BD Pharmingen	2138711	44 min	Optiview DAB IHC	Yes	N	DAB
123	HIER	15 MIN	ES05	PREDILUTE	DAKO	10096234	20 MIN	Flex+20mouse	N	N	DAB
124	cc1	56	epr3894	1/200	Abcam	YH102005C	32	opti view	n	n	DAB
125	ER2-20	20 min	ES05	1/100	DAKO	10092577	15 min	Bond Polymer Refine detection	N	Y	DAB
126	Pressure Cooker	8 mins	ES05	1:50	Dako	10092577	30 mins	Thermo Quanto Small Polymer	Yes	No	DAB
138	HIER HipH 9.0	20	ES05	RTU	Dako	10096234	20	Polymer - EnVision FLEX+	Y	N	DAB
141	HIER	20 mins	ES05	1:50	Dako	10086379	20 min	polymer	Y	N	DAB
144	Ultra CC1	16 min	M1	Pre-Dilute	Cell Marque	E06127	56 min	Optiview	No	Yes, copper	DAB
145	CC1	56	G168-728	1/40	CELL MARQUE	1315706A	28	VENTANA XT OPTIVIEW ihc v4	N	N	DAB
149	PT Link 97 C high pH	20	ES05	RTU	Dako	10089889	30	EnVison Flex	No	No	DAB
160	CC1	76MIN 100Å°C	545M1	prediluted	VENTANA	E07896	24MIN	ULTRAVIEW	N	CUSO4	DAB
164	ultraCC1	16	M1	predilute	Ventana	E01437	32	Optiview	N	N	DAB
170	PT Link Dako	20 min	ES05	ready to use	Dako	10083784	20 min	Flex EnVision Dako	N	N	DAB
175	HIER	32 min	M1	Predilute	Roche	E70276	60 min	Opti	No	Yes	DAB
181	Tris/EDTA pH 9	20	ES05	1:50	DAKO	10086379	20	DAKO EnvisionFex+	y	n	DAB
184	Hi pH Target Retrieval	20	ES05	RTU	Dako	10095903	20	FLEX+	N	N	DAB+
186	HIER EDTA buffer	20'	G168-728	1:50	Cell Marque	1315706A	15'	Bond Refine polymer detection kit	N	N	DAB
189	Ventana CC1	64 min.	M1	pre-dilute	Ventana	unknown	16 min	Ultraview	N	N	Ultraview DAB
191	CC1	60'	E505	1/10	novocastra	6025230	32'	ultraview DAB	Y	N	DAB
200	HIER	15	G168-728	1:400	Cell Marque	1423111C	10	HiDef (HRP)	N	N	DAB
202	Leica ER2	20 min	ES05	1/10	BD PHARMAGEN	4279889	15 MIN RT	LEICA REFINE DETECTION KIT	N	N	DAB
207	heat retrieval-Citrate	118 degres for 2 min (cool down 15 min)	G168-15	1/50	Biocare	110314	60 min	Mach 3	N	Y	DAB
210	TRS pH High	10	ES05	RTU	DAKO	10096234	30	Envison FLEX	Y	N	DAB
215	CC1	64	G168-15	1:30	Biocare	73013	60	Optiview	N	N	DAB
217	CC1 Ventana Ultra	64	M1	pre-dilute	Ventana	E03834	60	Optiview	N	copper	DAB
220	CC1	72	ES05	n	Dako	n	60	OptiView	N	N	DAB
221	pH9 Tris/EDTA	20 Minutes	ES05	1:25	Dako	62414	30	Mouse EnVision	Y	N	DAB

Table S2. Reported PMS2 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ab Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 MINUTES	EP51	1:15	Epitomics	EL090506	32 minutes	OptiView	N	COPPER	DAB
102	DAKO HIGH pH TARGET RETREIVAL	10/20/10	EP51	1/25	DAKO	10090994	30* RT	DAKO FLEX+	NO	CUSO4	DAB+
106	TRIS HCl microwave pressure	30	MRQ-28	1:15	Cell Marque	1430403-B	45	MACH4	N	N	DAB
107	Ventana CC1	40	EPR3947	Pre-diluted	Ventana	1417001C	48	OptiView DAB	Y	Y (Copper)	DAB
109	ULTRA CC1 at 100 degrees	64 min	EPR3947	RTU	CELL MARQUE	1329701D	20 MIN	OPTIVIEW	YES	YES	DAB
110	HEIR	20 mins	EP51	1:50	DAKO	10088744	30 mins	Polymer	N	N	DAB
111	CC1	36 min	EPR3947	Predilute	Ventana/Roche	1329701C	32 min	Ultraview	Yes	Copper	DAB
112	BOND Epitope Retrieval 2	40 minutes	EP51	1:25 Background Reducing Diluent "DAKO"	Cedarlane	EL021804	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	20min	PMS2	1/50	BD Biosciences	4154997	30min	Polymer	N	N	Dab
114	CC1	32min	EP51	1:25	Epitomics	EL050802	16min	Optiview	N	Copper	DAB
115	Target retrieval enV flex Trs High pH, 97C	30min	EP51	prediluted	Dako	10092835	30min	envison flex	N	N	enV Flex substrate solution 5min
116	CC1	64 min	EPR3947	RTU	Ventana	1417001B	60 min	Optiview DAB IHC	Yes	N	DAB
123	HIER	15 min	A16-4	1/50	BDBiosciences	4154997	30 min	Flex+30mouse	N	N	DAB
124	CC1	32	EP51	1/40	Dako	10096211	32	Opti View	n	n	DAB
125	ER2-20	20 min	A16-4	1/75	BD Biosciences	4154997	15 min	Bond Polymer Refine detection	N	Y	DAB
126	Pressure Cooker	30 mins	EP51	1:25	Dako	10092057	30 mins	Thermo Quanto Small Polymer	Yes	No	DAB
138	HIER pH 9.0	20	EP51	RTU	Dako	10091121	30	Polymer Envison FLEX	N	N	DAB
141	HIER	20 mins	EP51	1:50	Dako	10088744	30 min	Polymer	N	N	DAB
144	Ultra CC1	64 min	EPR3947	Pre-Dilute	Cell Marque	1417001B	60 min	Optiview	No	Yes, copper	DAB
145	CC1	56	A16-4	1/50	BIOCARE	51713	36	VENTANA XT OPTIVIEW ihc v4	N	N	N
149	PT Link 97 C high pH	20	EP51	RTU	Dako	10092835	20	EnVison Flex	Yes	No	DAB
160	CC1	92MIN 100°C	ER3947	PREDILUTED	VENTANA	1417001D	2H	ULTRAVIEW	Y	COPPER(CuS)	DAB
164	ultraCC1	56	EPR3947	predilute	Ventana	1417001B	32	Optiview	N	N	N
170	PT Link Dako	20 min	EP51	ready to use	Dako	10093476	20 min	Flex EnVision Dako	N	N	DAB
175	HIER	64 min	EPR3947	Predilute	Roch	1417001C	2 hours	Opti	No	Yes	DAB
181	tris/EDTA pH 9	20	EP51	1:50	Dako	10088744	30	Dako Envison Flex+	n	n	DAB
184	Hi pH Target Retrieval	20	EP51	RTU	Dako	10096479	20	FLEX	N	N	DAB+
186	HIER EDTA BUFFER	20'	ERP3947	1:2	Cell Marque	1417001A	15'	Bond Refine detection system	N	N	DAB
189	Ventana CC1	64 min.	EPR3947	pre-dilute	Ventana	unknown	32 min.	Ultraview	Y	N	Ultraview DAB
191	CC1	64'	EPR 3947	RTU	Roche	1417001c	48'	optiview	Y	N	DAB
200	HIER	15	MRQ-28	1:200	Cell Marque	1418103B	10	HiDef (HRP)	N	N	DAB
202	Leica ER2	30 MIN	A16-4	1/25	BD PHARMAGEN	4154997	15 MIN RT	LEICA REFINE DETECTION KIT	N	N	DAB
207	heat retrievevel	2 min 118 degrees	A16-4	1/50	becton Dickson	4154997	60 min	mach 3	N	Y	DAB
210	FLEX TRS High	10	EP51	RTU	DAKO	10093476	30	Envison FLEX	N	N	DAB
215	CC1	64	EPR3947	Predilute	Roche/Ventana	1329701C	40	Optiview	N	N	DAB
217	CC1 Ventana Ultra	92	EPR 3947	Pre-dilute	Ventana	1316203D	96	Optiview	N	copper	DAB
220	CC1	64	EPR3947	n	n	n	72	OptiView	N	N	DAB
221	pH9 Tris/EDTA	20	EP51	1:25	Biocare	10815	30	Rabbit EnVision	Y	N	DAB

Table S3. Reported MSH2 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	G219-1129	1:500	Cell Marque	1313003B	32 minutes	OptiView	N	COPPER	DAB
102	DAKO HIGH pH TARGET RETREIVAL	10/20/10	FE11	1/50	DAKO	10083837	30" RT	DAKO FLEX+	NO	CUSO4	DAB+
106	THIS HCl microwave pressure	30	SP93	1:15	Cell Marque	14014802B	45	MACH4	N	N	DAB
107	Ventana CC1	32	G219-1129	1:200	Cell Marque	1334002A	32	Optiview DAB	N	Y (Copper)	DAB
109	ULTRA CC1 at 100 degrees	32 min	G219-1129	RTU	CELL MARQUE	1334003C	8 MIN	OPTIVIEW	NO	YES	DAB
110	HEIR	20 mins	FE11	1:150	DAKO	10087780	20 min	Polymer	Y	N	DAB
112	BOND Epitope Retrieval 2	10 minutes	FE11	1:50 Renoir Red diluent	Biocare Medical	120213	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	20min	2SD12	1/25	Leica	6023005	30min	Polymer	N	N	Dab
114	CC1	32min	G219-1129	1:1000	Cell Marque	1313003F	16min	Optiview	N	Copper	DAB
115	Target retrieval enV flex Trs High pH, 97C	30min	FE11	prediluted	Dako	10092042	20min	envision flex	N	N	enV Flex substrate solution 5min
116	CC1	40 min	G219-1129	1/400	Cell Marque	1313003 B	48 min	Optiview DAB IHC	Non	N	DAB
123	HIER	15 min	G219-1129	1/100	BDBiosciences	4275638	20 min	Flex 30	N	N	DAB
124	cc1	30	G219-1129	PrA@-diluA@	Cell Marque	1417104C	12	iview	n	n	DAB
125	ER2-30	30 min	25D12	predilute	Novocastra/Leica	26872	15 min	Bond Polymer Refine detection	N	Y	DAB
126	Pressure Cooker	8 mins	FE11	1:75	Dako	10092706	30 mins	Thermo Quanto Small Polymer	Yes	No	DAB
138	HIER pH 9.0	20	FE11	RTU	Dako	10089129	20	Polymer - Envision FLEX+	Y	N	DAB
141	HIER	20 mins	FE11	1:150	Dako	10087780	20 min	Polymer	Y	N	DAB
144	Ultra CC1	16 min	G219-1129	Pre-Dilute	Cell Marque	1313004C	16 min	Optiview	No	Yes, copper	DAB
145	CC1	32	G219-1129	1/100	CELL MARQUE	1313003A	8	VENTANA XT OPTIVIEW ihc v4	N	N	DAB
149	PT Link 97 C high pH	20	FE11	RTU	Dako	10088045	20	EnVision Flex	Yes	No	DAB
160	CC1	64MIN 100A°C	G219-1129	PREDILUTED	VENTANA	1417104D	48MIN	ULTRAVIEW	N	COPPER(CuS)	DAB
164	ultraCC1	16	G219-1129	predilute	Ventana	1417104B	16	Optiview	N	N	DAB
170	PT Link Dako	20 min	FE11	ready to use	Dako	10090394	20 min	Flex EnVision Dako	N	N	DAB
175	HIER	32 min	9129-1129	Predilute	Roche	417104D	32 min	Opti	No	Yes	DAB
181	pH9 Tris/EDTA	20	FE11	1:150	Dako	10087780	20	Dako Envision Flex+	y	n	DAB
184	Hi pH Target Retrieval	20	FE11	RTU	Dako	10096663	20	FLEX+	N	N	DAB+
186	HIER EDTA BUFFER	20'	G219-1129	1:200	Cell Marque	1303003A	15'	Bond Refine detection kit	N	N	DAB
189	Ventana CC1	32 min.	G219-1129	pre-dilute	Cell Marque	unknown	16 min.	Ultraview	N	N	Ultraview DAB
191	CC1	32'	G219-1129	RTU	Roche	1417104c	16'	optiview	N	N	DAB
200	HIER	15	G219-1129	1:200	Cell Marque	1417104L	10	HiDef (HRP)	N	N	DAB
202	Leica ER2	30 MIN	25D12	RTU	LEICA	28250	15 MIN RT	LEICA REFINE DETECTION KIT	N	N	DAB
207	heat retrievei	20 min at 118 degrees	FE11	1/4000	calbiochem	34525	60 min	Mach 3	N	Y	DAB
210	FLEX TRS High	10	FE11	RTU	DAKO	10093148	30	Envision FLEX	N	N	DAB
215	CC1	24	G219-1129	Predilute	Cell Marque	1313004L	16	Optiview	N	N	DAB
217	CC1 Ventana Ultra	56	G219-1129	pre-dilute	Ventana	1313004C	32	Optiview	N	copper	DAB
220	CC1	32	G219-1129	n	CellMarque	n	32	OptiView	N	N	DAB
221	pH9 Tris/EDTA	30	FE11	1:25	Biocare	92214	30	Mouse EnVision	Y	N	DAB

Table S4. Reported MSH6 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	32 minutes	EP49	1:200	Epitomics	CJ052301L	32 minutes	OptiView	N	COPPER	DAB
102	DAKO FLEX HIGH pH TARGET RETRIEVAL	10/20/10	EP49	1/100	DAKO	10079041	30"	DAKO FLEX+	NO	CUSO4	DAB+
106	TRIS HCl microwave pressure	30	25D12	1:50	Novacastra	6023005	45	MACH4	N	n	DAB
107	Ventana CCl	60	44/MSH6	1:300	BD Biosystems	3200774	32	Ultraview DAB	N	Y (Copper)	DAB
109	ultra CC1 at 100 degrees	64 min	44	RTU	VENTANA	E02714	20 MIN	OPTIVIEW	NO	YES	DAB
110	HEIR	20 mins	EP49	1:300	DAKO	10088444	30 mins	Polymer	N	N	DAB
111	CC1	64 min	BC/44	Predilute	Biocare Medical	61214	32 min	Ultraview	N	Copper	DAB
112	BOND Epitope Retrieval 2	40 minutes	EP49	1:1500	Cedarlane	CJ052301	30 minutes	BOND Polymer Refine	none	none	DAB
113	High pH	20min	EP49	Predilute	Dako	10095909	15min	Polymer	N	N	Dab
114	CC1	32min	EP49	1:200	Epitomics	C1090101	16min	Optiview	N	Copper	DAB
115	Target retrieval enV flex Trs High pH, 97C	30min	EP49	prediluted	Dako	10092289	20min	envison flex	N	N	enV Flex substrate solution 5min
116	CC1	32 min	BC/44	1/200	Biocare Medical	31313	48 min	Optiview DAB IHC	NO	N	DAB
123	HIER	15 min	EPR3945	1/200	Cedarlane/ABCAM	GR189185-5	40 min	flex+20rabbit	N	N	DAB
124	CC1	32	EPR3945	1/100	Abcam	GR177321-7	32	Opti View	n	n	Dab
125	ER2-20	20 min	44/MSH6	1/1500	BD Biosciences	11917	15 min	Bond Polymer Refine detection	N	Y	DAB
126	Pressure Cooker	8 mins	EP49	1:75	Dako	10092708	30 mins	Thermo Quanto Small Polymer	Yes	No	DAB
138	High pH 9.0	20	EP49	RTU	Dako	10095022	20	Polymer - Envision FLEX	N	N	DAB
141	HIER	20 mins	EP49	1:300	Dako	10088444	30 mins	Polymer	N	N	DAB
144	Ultra CC1	8 min	EP49	1:200	Epitomics	EL061801	32 min	Optiview	No	Yes, copper	DAB
145	CC1	32	44	1/300	CELL MARQUE	1313501A	24	VENTANA XT OPTIVIEW ihc v4	N	N	DAB
149	PT Link 97 C high pH	20	EP49	RTU	Dako	10088047	20	EnVision Flex	No	No	DAB
160	CC1	92MIN 100°C	44	PREDILUTED	VENTANA	E07629	1H	ULTRAVIEW	N	COPPER	DAB
164	ultraCC1	16	44	predilute	Ventana	E02714	32	Optiview	N	N	DAB
170	PT Link Dako	20 min	EP49	ready to use	Dako	10092289	20 min	Flex Envision Dako	N	N	DAB
175	HIER	64 min	H4	1:100	Cell Marque	1112208C	32 min	Opti	No	Yes	DAB
181	Tris/EDTA pH 9	20	EP49	1:300	Dako	10088444	30	Dako Envision Flex+	n	n	DAB
184	Hi pH Target Retrieval	20	EP49	RTU	Dako	10095909	20	FLEX	N	N	DAB+
186	HIER CITRATE BUFFER	20'	BC/44	1:50	Biocare Medical	11714	15'	Bond refine detection system	N	N	DAB
189	Ventana CC1	64 min.	44	pre-dilute	Ventana	unknown	16 min.	Ultraview	N	N	Ultraview DAB
191	CC1	30'	44	1/800	BD Biosciences	25795	32'	ultraview DAB	Y	N	DAB
200	HIER	15	44	1:500	Cell Marque	1416302A	10	HiDef (HRP)	N	N	DAB
202	Leica ER2	40 MIN	PU29	1/25	ABCAM	GR189185	15 MIN RT	LEICA REFINE DETECTION KIT	N	N	DAB
207	heat retrieval-citrate	118 for 2 min	44	1/400	becton Dickson	4050545	60 min	mach 3	N	Y	DAB
210	FLEX TRS High	10	EP49	RTU	DAKO	10095022	20	Envision FLEX	N	N	DAB
215	CC1	56	EPR3945	1:100	Ab-Cam	GR129623-8	32	Optiview	N	N	DAB
217	CC1 Ventana Ultra	64	44/MSH6	1:3000	BD	51217	60	Optiview	N	copper	DAB
221	pH9 Tris/EDTA	20 Minutes	EP49	1:50	Biocare	20215	30	Rabbit EnVision	Y	N	DAB

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	29	86.21	25	25/25 (100%)	1	1	1	1	1
102	29	93.1	27	27/27 (100%)	1	1	1	1	1
106	29	93.1	27	27/27 (100%)	1	1	1	1	1
107	29	93.1	27	26/27 (96%)	1	0.86	0.95	1	0.9
109	29	93.1	27	26/27 (96%)	1	0.86	0.95	1	0.9
110	29	93.1	27	27/27 (100%)	1	1	1	1	1
111	29	86.21	25	24/25 (96%)	1	0.83	0.95	1	0.88
112	29	89.66	26	26/26 (100%)	1	1	1	1	1
113	29	93.1	27	27/27 (100%)	1	1	1	1	1
114	29	93.1	27	27/27 (100%)	1	1	1	1	1
115	29	86.21	25	25/25 (100%)	1	1	1	1	1
116	29	93.1	27	27/27 (100%)	1	1	1	1	1
123	29	96.55	28	28/28 (100%)	1	1	1	1	1
124	29	96.55	28	21/28 (75%)	1	0.13	0.74	1	0.17
125	29	96.55	28	27/28 (96%)	0.95	1	1	0.89	0.92
126	29	100	29	29/29 (100%)	1	1	1	1	1
138	29	93.1	27	27/27 (100%)	1	1	1	1	1
141	29	96.55	28	28/28 (100%)	1	1	1	1	1
144	29	93.1	27	25/27 (93%)	1	0.71	0.91	1	0.79
145	29	89.66	26	24/26 (92%)	1	0.67	0.91	1	0.75
149	29	86.21	25	25/25 (100%)	1	1	1	1	1
160	29	93.1	27	27/27 (100%)	1	1	1	1	1
164	29	96.55	28	28/28 (100%)	1	1	1	1	1
170	29	96.55	28	28/28 (100%)	1	1	1	1	1
175	29	93.1	27	27/27 (100%)	1	1	1	1	1
181	29	93.1	27	27/27 (100%)	1	1	1	1	1
184	29	96.55	28	28/28 (100%)	1	1	1	1	1
186	29	82.76	24	24/24 (100%)	1	1	1	1	1
189	29	89.66	26	26/26 (100%)	1	1	1	1	1
191	29	86.21	25	25/25 (100%)	1	1	1	1	1
200	29	100	29	11/29 (38%)	0.14	1	1	0.31	0.08
202	29	86.21	25	23/25 (92%)	1	0.71	0.9	1	0.78
207	29	89.66	26	26/26 (100%)	1	1	1	1	1
210	29	96.55	28	28/28 (100%)	1	1	1	1	1
215	29	89.66	26	26/26 (100%)	1	1	1	1	1
217	29	96.55	28	28/28 (100%)	1	1	1	1	1
220	29	93.1	27	27/27 (100%)	1	1	1	1	1
221	29	89.66	26	26/26 (100%)	1	1	1	1	1

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

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	28	82.14	23	23/23 (100%)	1	1	1	1	1
102	28	92.86	26	26/26 (100%)	1	1	1	1	1
106	28	96.43	27	27/27 (100%)	1	1	1	1	1
107	28	100	28	28/28 (100%)	1	1	1	1	1
109	28	96.43	27	27/27 (100%)	1	1	1	1	1
110	28	96.43	27	27/27 (100%)	1	1	1	1	1
111	28	96.43	27	27/27 (100%)	1	1	1	1	1
112	28	100	28	28/28 (100%)	1	1	1	1	1
113	28	96.43	27	27/27 (100%)	1	1	1	1	1
114	28	92.86	26	26/26 (100%)	1	1	1	1	1
115	28	89.29	25	25/25 (100%)	1	1	1	1	1
116	28	96.43	27	27/27 (100%)	1	1	1	1	1
123	28	96.43	27	26/27 (96%)	1	0.86	0.95	1	0.9
124	28	92.86	26	26/26 (100%)	1	1	1	1	1
125	28	92.86	26	26/26 (100%)	1	1	1	1	1
126	28	100	28	28/28 (100%)	1	1	1	1	1
138	28	92.86	26	26/26 (100%)	1	1	1	1	1
141	28	96.43	27	26/27 (96%)	1	0.88	0.95	1	0.91
144	28	92.86	26	26/26 (100%)	1	1	1	1	1
145	28	92.86	26	26/26 (100%)	1	1	1	1	1
149	28	89.29	25	25/25 (100%)	1	1	1	1	1
160	28	92.86	26	26/26 (100%)	1	1	1	1	1
164	28	96.43	27	27/27 (100%)	1	1	1	1	1
170	28	96.43	27	27/27 (100%)	1	1	1	1	1
175	28	92.86	26	26/26 (100%)	1	1	1	1	1
181	28	85.71	24	24/24 (100%)	1	1	1	1	1
184	28	85.71	24	24/24 (100%)	1	1	1	1	1
186	28	92.86	26	26/26 (100%)	1	1	1	1	1
189	28	89.29	25	24/25 (96%)	1	0.86	0.95	1	0.9
191	28	92.86	26	26/26 (100%)	1	1	1	1	1
200	28	100	28	23/28 (82%)	0.8	0.88	0.94	0.64	0.61
202	28	85.71	24	24/24 (100%)	1	1	1	1	1
207	28	92.86	26	26/26 (100%)	1	1	1	1	1
210	28	92.86	26	25/26 (96%)	1	0.88	0.95	1	0.91
215	28	85.71	24	24/24 (100%)	1	1	1	1	1
217	28	92.86	26	26/26 (100%)	1	1	1	1	1
220	28	96.43	27	27/27 (100%)	1	1	1	1	1
221	28	100	28	28/28 (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	31	87.1	27	27/27 (100%)	1	1	1	1	1
102	31	83.87	26	26/26 (100%)	1	1	1	1	1
106	31	90.32	28	28/28 (100%)	1	1	1	1	1
107	31	90.32	28	28/28 (100%)	1	1	1	1	1
109	31	93.55	29	29/29 (100%)	1	1	1	1	1
110	31	90.32	28	28/28 (100%)	1	1	1	1	1
112	31	90.32	28	28/28 (100%)	1	1	1	1	1
113	31	93.55	29	29/29 (100%)	1	1	1	1	1
114	31	87.1	27	27/27 (100%)	1	1	1	1	1
115	31	87.1	27	27/27 (100%)	1	1	1	1	1
116	31	96.77	30	30/30 (100%)	1	1	1	1	1
123	31	93.55	29	29/29 (100%)	1	1	1	1	1
124	31	90.32	28	28/28 (100%)	1	1	1	1	1
125	31	90.32	28	28/28 (100%)	1	1	1	1	1
126	31	93.55	29	29/29 (100%)	1	1	1	1	1
138	31	90.32	28	28/28 (100%)	1	1	1	1	1
141	31	83.87	26	26/26 (100%)	1	1	1	1	1
144	31	90.32	28	28/28 (100%)	1	1	1	1	1
145	31	96.77	30	30/30 (100%)	1	1	1	1	1
149	31	93.55	29	29/29 (100%)	1	1	1	1	1
160	31	93.55	29	29/29 (100%)	1	1	1	1	1
164	31	93.55	29	29/29 (100%)	1	1	1	1	1
170	31	93.55	29	29/29 (100%)	1	1	1	1	1
175	31	93.55	29	29/29 (100%)	1	1	1	1	1
181	31	87.1	27	26/27 (96%)	1	0.67	0.96	1	0.78
184	31	96.77	30	30/30 (100%)	1	1	1	1	1
186	31	93.55	29	29/29 (100%)	1	1	1	1	1
189	31	77.42	24	24/24 (100%)	1	1	1	1	1
191	31	83.87	26	26/26 (100%)	1	1	1	1	1
200	31	93.55	29	16/29 (55%)	0.5	1	1	0.19	0.17
202	31	83.87	26	26/26 (100%)	1	1	1	1	1
207	31	87.1	27	27/27 (100%)	1	1	1	1	1
210	31	77.42	24	23/24 (96%)	1	0.67	0.95	1	0.78
215	31	87.1	27	27/27 (100%)	1	1	1	1	1
217	31	93.55	29	29/29 (100%)	1	1	1	1	1
220	31	87.1	27	27/27 (100%)	1	1	1	1	1
221	31	90.32	28	28/28 (100%)	1	1	1	1	1

Table S8. Descriptive statistics for MSH6 based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	31	87.1	27	27/27 (100%)	1	1	1	1	1
102	31	90.32	28	27/28 (96%)	1	0.67	0.96	1	0.78
106	31	93.55	29	29/29 (100%)	1	1	1	1	1
107	31	96.77	30	30/30 (100%)	1	1	1	1	1
109	31	93.55	29	29/29 (100%)	1	1	1	1	1
110	31	87.1	27	27/27 (100%)	1	1	1	1	1
111	31	90.32	28	28/28 (100%)	1	1	1	1	1
112	31	96.77	30	30/30 (100%)	1	1	1	1	1
113	31	90.32	28	28/28 (100%)	1	1	1	1	1
114	31	90.32	28	28/28 (100%)	1	1	1	1	1
115	31	80.65	25	25/25 (100%)	1	1	1	1	1
116	31	93.55	29	29/29 (100%)	1	1	1	1	1
123	31	90.32	28	28/28 (100%)	1	1	1	1	1
124	31	87.1	27	27/27 (100%)	1	1	1	1	1
125	31	87.1	27	27/27 (100%)	1	1	1	1	1
126	31	93.55	29	29/29 (100%)	1	1	1	1	1
138	31	90.32	28	28/28 (100%)	1	1	1	1	1
141	31	93.55	29	29/29 (100%)	1	1	1	1	1
144	31	90.32	28	27/28 (96%)	0.96	1	1	0.75	0.84
145	31	90.32	28	28/28 (100%)	1	1	1	1	1
149	31	93.55	29	29/29 (100%)	1	1	1	1	1
160	31	93.55	29	29/29 (100%)	1	1	1	1	1
164	31	90.32	28	28/28 (100%)	1	1	1	1	1
170	31	93.55	29	29/29 (100%)	1	1	1	1	1
175	31	93.55	29	29/29 (100%)	1	1	1	1	1
181	31	87.1	27	27/27 (100%)	1	1	1	1	1
184	31	87.1	27	27/27 (100%)	1	1	1	1	1
186	31	77.42	24	24/24 (100%)	1	1	1	1	1
189	31	93.55	29	29/29 (100%)	1	1	1	1	1
191	31	74.19	23	23/23 (100%)	1	1	1	1	1
200	31	100	31	28/31 (90%)	0.89	1	1	0.5	0.62
202	31	70.97	22	21/22 (95%)	1	0.5	0.95	1	0.65
207	31	87.1	27	27/27 (100%)	1	1	1	1	1
210	31	93.55	29	29/29 (100%)	1	1	1	1	1
215	31	90.32	28	28/28 (100%)	1	1	1	1	1
217	31	87.1	27	27/27 (100%)	1	1	1	1	1
221	31	100	31	31/31 (100%)	1	1	1	1	1