Mismatch Repair Protein antibodies and their performance in the UK National External Quality Assessment Scheme for Immunocytochemistry and In-situ Hybridisation

UK NEQAS
International Quality Expertise

Suzanne Parry and Andrew Dodson. UK NEQAS ICC & ISH, London, UK (sparry@ukneqasiccish.org)

BACKGROUND

DNA mismatch repair (MMR) proteins correct errors in the DNA code that occur when it is copied at mitosis. Loss-of-function mutations in any one of the four MMR proteins (MLH1, MSH2, MSH6, PMS2) leads to failure of the whole system. Errors accumulate and the risk of cancer increases significantly, particularly the risk of colorectal (CRC) and endometrial carcinomas.

Constitutional pathogenic MMR protein mutations cause Lynch syndrome (LS), which is an important hereditary disease responsible for >3% of CRC's. Loss of expression of one or more MMR protein in a patient's tumour cells is highly suggestive of LS [1]. Testing for loss of expression is usually done by immunohistochemistry (IHC).

UK NEQAS ICC & ISH (https://ukneqasiccish.org/) has conducted EQA on MMR protein IHC since 2011. We examined our data, looking for significant performance trends over time and any associations between assessment score and methodological parameters.

MATERIALS and METHODS

Four EQA runs were carried-out per year. At each, participants were asked to demonstrate two of the four MMR proteins by IHC, using their routine clinical testing procedures. The protein pairs requested (MLH1/PMS2 and MSH2/MSH6) alternated between runs; patent and deficient tumour appropriate to the requested pair were also distributed.

Assessment of technical quality in the returned stained slides was done by four expert assessors working independently to an agreed pre-specified set of standards. Dependent on overall quality, they individually awarded a mark in the range 1–5, (see Table 1).

Data on primary antibody clone, IHC detection system and automated staining platform used were collated for EQA runs conducted between 2011 and 2019.

Results and statistical analyses are presented for these. Data were curated in Excel (Microsoft) and analysed in Prism (Graphpad).

Mark	Quality descriptor
1	**Unacceptable**. No reliable information can be obtained.
2	**Unacceptable**. Clinically incorrect staining in at least one tumour.
3	**Acceptable**. Borderline clinically appropriate staining. Significant improvement required.
4	**Acceptable**. Clinically appropriate staining. Minor technical issue(s).
5	**Acceptable**. Optimal staining.
Final score	**Final assessed category**
4 to 9	Unacceptable (**FAIL**).
10 to 12	Borderline acceptable (**PASS**).
13 to 20	Acceptable (**PASS**).

TABLE 1. Scoring guidelines. The four assessors' marks were summed to give the participant's final score (range: 4-20).

RESULTS

For each of the MMR proteins 14 assessment runs were conducted. A total of 4447 participant submissions were received, Table 2 gives further descriptive statistics. Table 3 and Chart 1 relate information about the success of participants in producing 'Acceptable' staining at each assessment run for each of the MMR proteins.

MMR protein	Per run (N)		Total (N)
	Median	IQR	
MLH1	76	70-88	1108
MSH2	78	70-87	1112
MSH6	79	70-88	1117
PMS2	78	70-90	1110
Total			**4447**

TABLE 2. Number of submissions at each MMR protein assessment run. IQR: inter-quartile range.

MMR protein	Acceptable (%)			Trend sig.?
	Overall	First run	Last run	
MLH1	88.8%	75.0%	96.2%	Yes
MSH2	87.6%	87.9%	97.0%	Yes
MSH6	81.8%	82.1%	85.1%	Yes
PMS2	86.8%	82.1%	92.5%	No

TABLE 3. Proportions obtaining an 'Acceptable' score (≥10). Trend for increase in proportion obtaining an 'Acceptable' score over time was significant for MLH1, MSH2 and MSH6 (P<0.0005), but not for PMS2 (P<0.05).

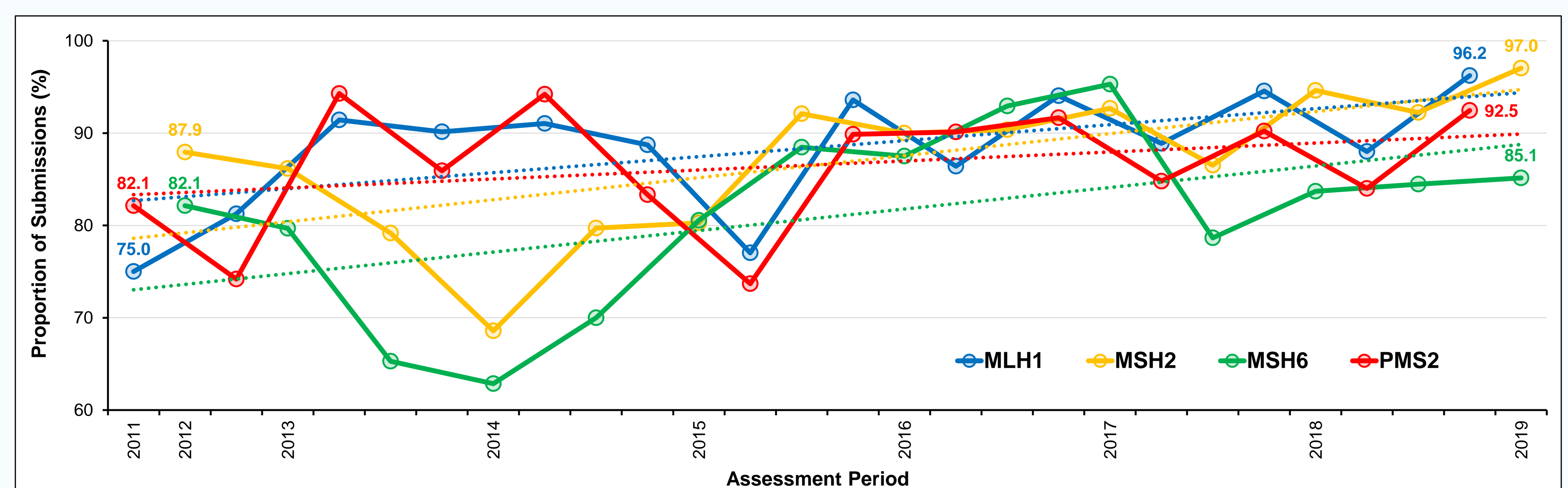


CHART 1. Proportion of 'Acceptable' submissions at each run within each arm of the MMR Module over the period examined. The dotted-lines indicate linear trend lines. Data labels are shown for first and last runs.

Primary antibodies: Three different clones were used for the demonstration of MLH1 and MSH2 proteins respectively. And for MSH6 and PMS2 proteins, four. There were considerable differences in their performance as assessed by average score achieved at assessment (see Table 4 and Chart 2). Proportional usage for the different clones is shown in Charts 3 a-d. For MLH1, MSH2 and MSH6 there has been an increase in the use of clones showing better performance at assessment with a concomitant decline in the use of less successful clones. This has not been seen in the case of PMS2.

Antigen	Antibody clone	Submissions		Average score	
		Count	Proportion	Overall	IQR
MLH1	ES05	530	47.8%	14.5	13.7-15.0
	Clone M1	379	34.2%	14.7	13.5-14.9
	G168-15	130	11.7%	13.1	11.8-14.4
	Other/NS	69	6.2%	12.3	11.5-13.5
MSH2	G219-1129	581	52.4%	14.2	13.0-15.0
	FE11	312	28.2%	14.8	13.8-15.3
	25D12	172	15.5%	13.5	12.0-13.6
	Other/NS	47	4.2%	12.4	11.0-14.0
MSH2	Clone 44	479	43.2%	12.2	11.2-13.0
	EP49	416	37.5%	15.6	15.1-15.9
	PU29	83	7.5%	10.7	6.6-11.3
	SP93	70	6.3%	15.6	13.3-16.1
	Other/NS	69	6.2%	14.0	13.3-14.9
PMS2	EPR3947	435	39.3%	13.7	12.6-14.8
	EP51	321	29.0%	14.8	14.1-15.2
	A16-4	252	22.7%	14.7	14.2-15.1
	MOR4G	59	5.3%	11.7	9.0-12.9
	Other/NS	43	3.9%	12.5	11.6-13.4

TABLE 4. Primary antibody clones. Total number of submissions for each clone and the proportion that represented for any given MMR protein. Performance is indicated by average score achieved (see also Chart 2). Antibody clones used <50 times overall have been incorporated into 'Other/NS'; NS = not stated.

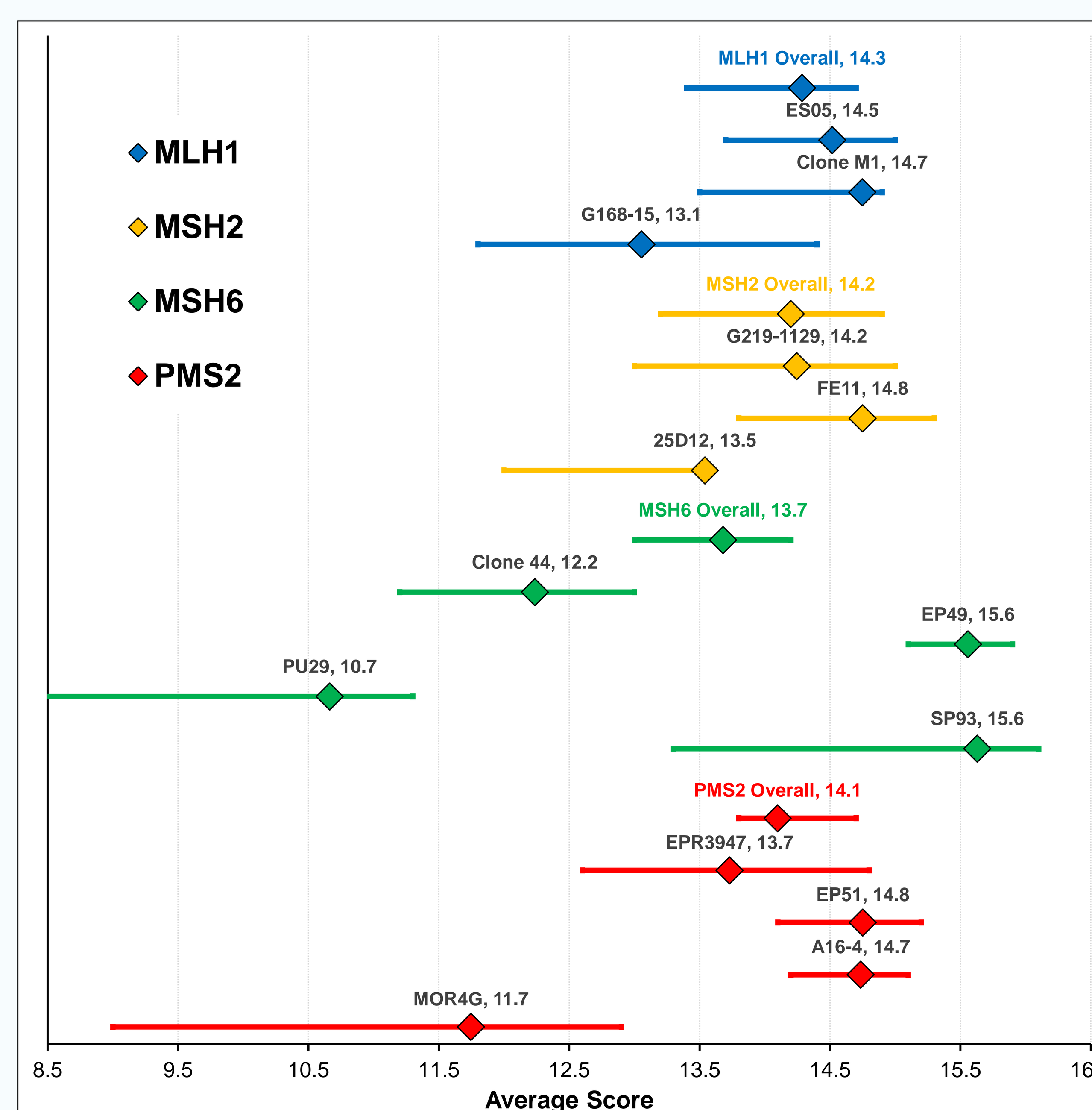
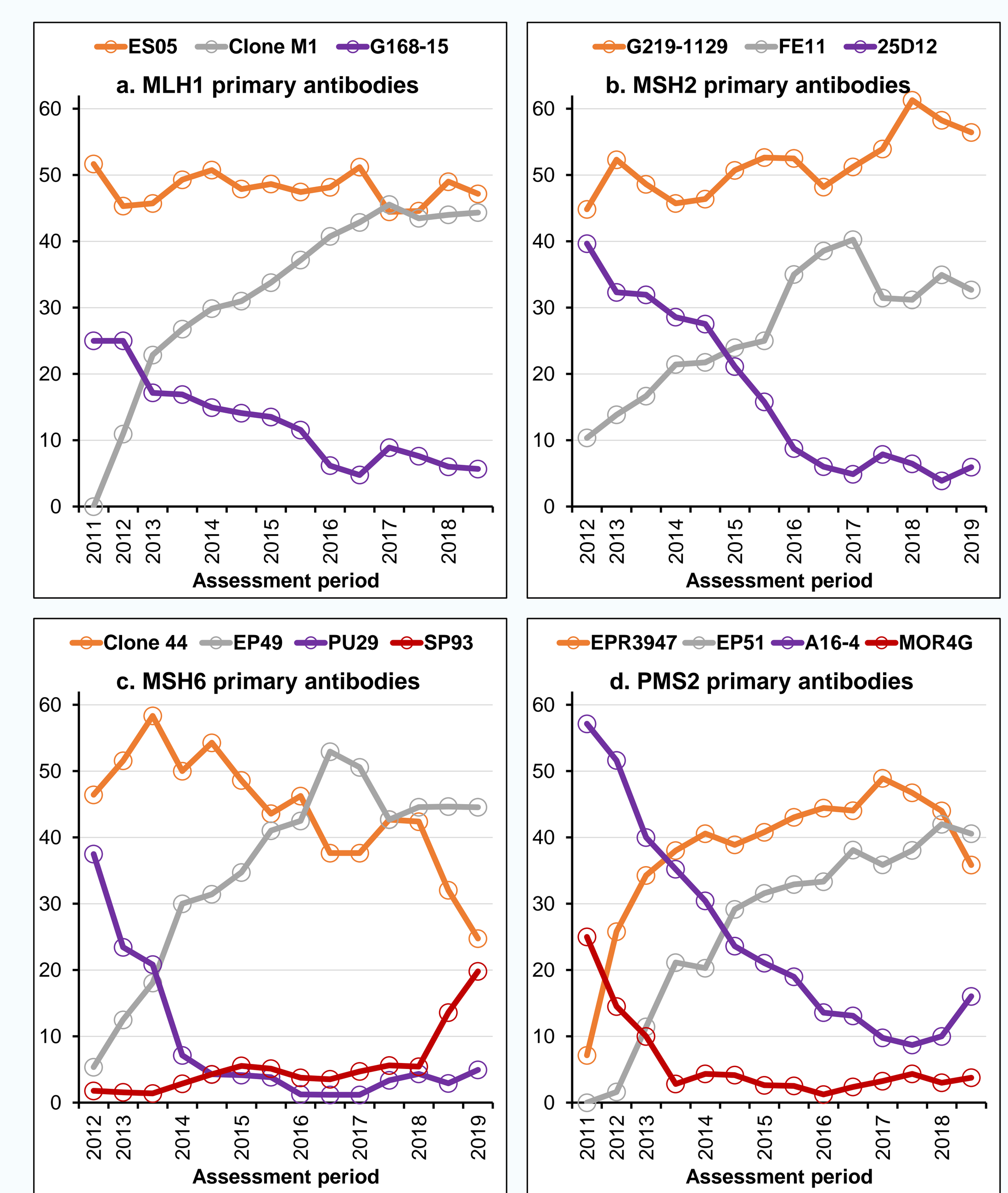


CHART 2. Forest plot illustrating primary antibody performance. Diamonds indicate average scores achieved by each clone over all assessments. Whiskers = IQR. Average scores overall for each MMR protein also shown; 'Other/NS' category not shown. Lower IQR for PU29 truncated.



CHARTS 3 a-d. Primary antibody clone usage for each MMR protein. In each chart the left-hand scale is proportional usage (%). 'Other/NS' category not shown.

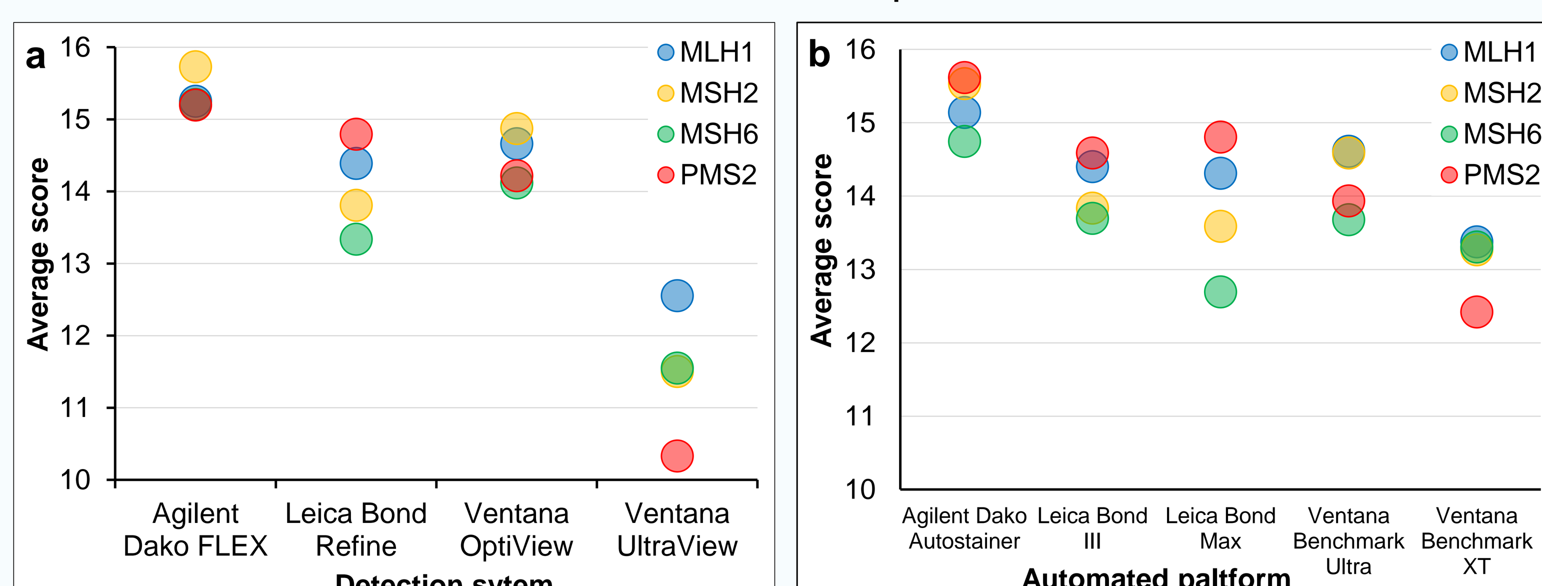
Detection systems and automated platforms: Four detection kits from three suppliers were used. They were associated with differences in performance as measured by average score achieved for all four MMR proteins. Five platforms from three suppliers were used. These were not associated with performance differences.

CHARTS 4 a & b.

a). Detection systems used. The circles indicate average score achieved by participant's using that detection system for the demonstration of each of the four MMR proteins

b). Automated platforms used. As for Detection systems the circles indicate average scores.

Detection systems and automated platforms used <50 times have not been shown. 'Other/NS' category not shown.



CONCLUSIONS

Performance in demonstration of MLH1, MSH2 and MSH2 has improved during the time course examined (2011 to 2019). That for PMS2 has not changed significantly.

There are significant difference in performance of clones.

Better performing clones for the demonstration of MLH1, MSH2 and MSH6 have increased in usage. This has not been the case for PMS2. This may explain the lack of improvement in results for this protein.

Agilent Dako's FLEX detection is associated with better scores, and Ventana UltraView worse scores in the demonstration of all four proteins.

Differences in performance is not platform dependent.

REFERENCE. 1. Molecular testing for Lynch syndrome in people with colorectal cancer: systematic reviews and economic evaluation. Snowsill T, et al. Health Technol Assess 2017;21(51).

31st European Congress of Pathology, 2019
Nice, France. PS-20-018

Information presented in this poster is subject to copyright and may not be reproduced without permission.

UK NEQAS ICC & ISH. Office 127, Finsbury Business Centre, 40 Bowling Green Lane, London EC1R 0NE UK

Contact Suzanne Parry:
sparry@ukneqasiccish.org