Factors associated with Ki-67 immunohistochemistry staining quality: findings from UK NEQAS ICC & ISH assessment data

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Lay Summary: Ki-67 is a protein present in dividing cells. In breast cancer, the proportion of cells expressing Ki-67 gives information about how aggressive a tumour is – how likely it is to spread to other parts of the body or to recur. Assessment of Ki-67 expression is affected by the quality of the immunohistochemistry method used. So, we examined method data obtained from more than 2,000 breast cancer samples and found that the primary antibody type and different factors in the method all had an effect. It is important to know these facts so that laboratories can choose primary antibodies and methods best suited to the staining equipment they have in order to produce the correct assessment of Ki-67 expression.

AIMS: To examine Ki-67 data collected by the UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation (UK NEQAS ICC & ISH) in the course of its routine external quality assessments (EQA's). Comparing the quality of staining produced by different commercially available primary antibody clones, and looking for associations between those clones and major immunohistochemical (IHC) methodological factors that significantly impacted on staining quality.

MATERIALS AND METHODS: The study examined data gathered from EQA runs conducted between July 2013 and April 2017 that examined the quality of Ki-67 staining.

At each run participants were provided with formalin fixed paraffin embedded (FFPE) sections, which they were required to stain for Ki-67 using their routine IHC methodology.

Stained slides were returned for central assessment by a panel of biomedical scientists and pathologists experienced in the assessment of IHC staining quality. Indicators of good staining quality included appropriate stain localisation (nuclear), absence of non-specific and inappropriate staining, but no formal quantitative assessment was undertaken.

A nominal scoring scale running between 4 and 20 was used to indicate staining quality, with scores of 4 indicating poor staining and scores of 20, excellent staining.

Methodological data were obtained from each participating centre at each submission, which included detailed information about:

- nature of primary antibody,
- IHC detection system,
- antigen retrieval method (if used),
- automated staining platform (if any).

RESULTS

EQA RUN STATISTICS: Eight EQA runs were carried out; four between July 2013 and April 2014, and four between July 2016 and April 2017.

- Median number of participating centres over the eight runs was 326,
- The range for number of participating centres was 299-348.

In total, 2,601 individual submissions were received:

- 1,398 (53.7%) from UK-based centres; 1,203 (46.3%) from centres outside the UK,
- 374 centres made at least one submission,
- 270 (72.2%) made submissions to all eight runs.

PRIMARY ANTIBODIES USED: In the case of more than 90% of all submissions, the primary antibody used was one of five clones as shown in the pie-chart and below (Figure1).

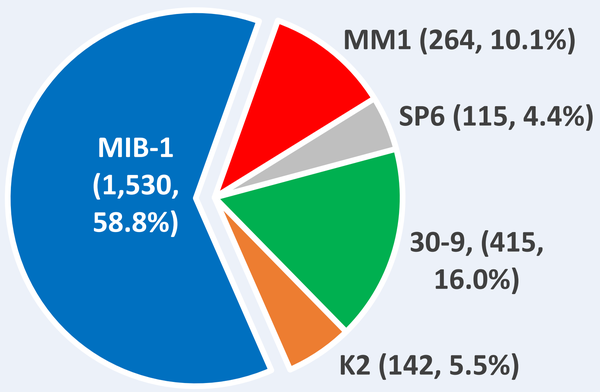

Figure 1. Primary antibody use. First figure in brackets after the clone name is count of submissions for that clone, the second is proportion of total submissions.

In addition to the five main clones, a variety of other clones were used accounting for 1.2% of submissions in aggregate (each of these was used on <100 occasions). In the remaining 4.0% of submissions the clone used was not stated.

With the exception of SP6, which was supplied by a variety of companies, primary antibodies were almost exclusively obtained from a single commercial supplier:

- 30-9 (CONFIRM 790-4286), 99.8% from Ventana Medical Systems Inc, Arizona USA,
- K2 (ACK02) and MM1 (Ki67-MM1-L), 100.0% and 97.3%, respectively, from Leica Biosystems, Buffalo Grove, Illinois USA,
- MIB1 (M7240), 99.9% from Agilent Dako, Santa Clara, California USA.

PRIMARY ANTIBODY PERFORMANCE: Descriptive statistics for primary antibody performance are shown below (Figure2 and Table 1).

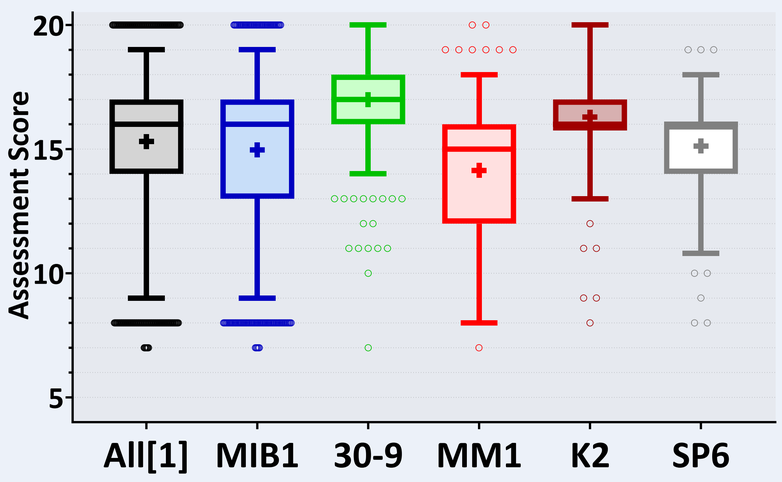

Figure 2. Box & whiskers plot of overall and primary antibody performance. Bounds of box are 25th and 75th quartiles, line within box represents median, the "+" symbol shows the mean, whiskers are 5th and 95th percentile range.

Table 1. Overall and primary antibody performance. Showing the data illustrated in Figure 2 and additional descriptive statistics for the whole cohort (All) and individual primary antibody groupings.

[Note 1] in both the Figure and the Table the 'All' group does not include 'Other (use<100)' or 'Not Stated' groups.

S.D. = standard deviation, S.E. mean= standard error of mean, CI = confidence interval

	All [1]	MIB1	30-9	MM1	K2	SP6
Number of values	2466	1530	415	264	142	115
25% Percentile	14	13	16	12	16	14
Median	**16**	**16**	**17**	**15**	**16**	**16**
75% Percentile	17	17	18	16	17	16
Mean	**15.3**	**15.0**	**17.0**	**14.1**	**16.3**	**15.1**
S.D.	2.8	2.9	1.9	3.0	2.1	2.2
S.E. mean	0.06	0.07	0.09	0.18	0.18	0.20
Lower 95% CI mean	15.2	14.8	16.8	13.8	15.9	14.7
Upper 95% CI mean	15.4	15.1	17.2	14.5	16.6	15.5

METHODOLOGICAL PARAMETER ASSOCIATED WITH PERFORMANCE: For each primary antibody clone independently, the data were examined for significant associations between methodological parameters and performance at assessment. Re-analysis of the primary antibody performance was done, taking into account the effects of methodological parameters that were found to have a significant effect.

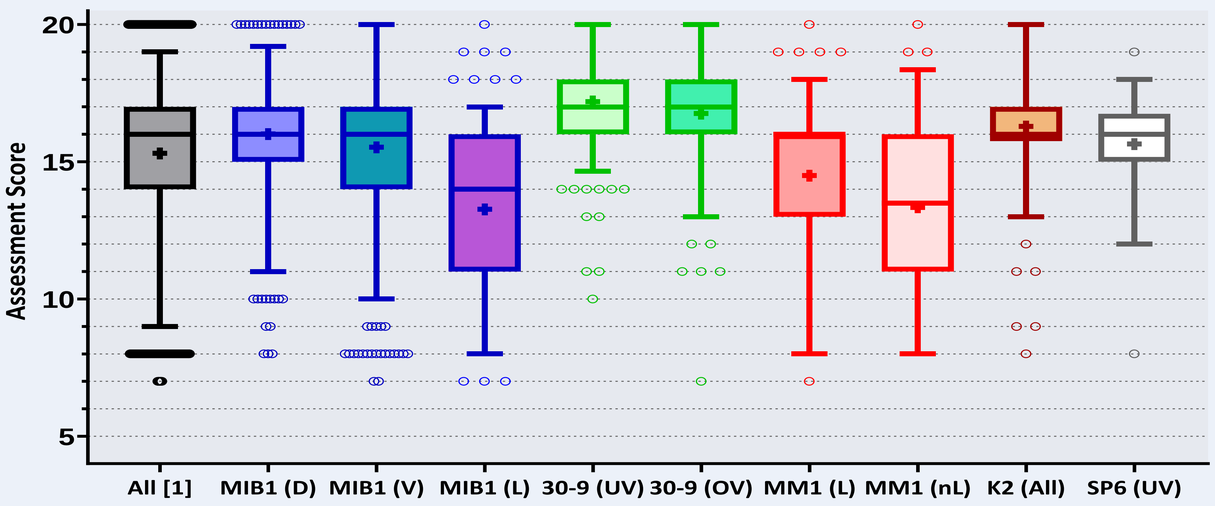

Figure 3. Box & whiskers plot of overall and optimised primary antibody performance. Box and whiskers description same as Figure 1. MIB1 (D) = MIB1 using Dako reagents/platform, MIB1 (V) = using Ventana reagents/platforms, MIB1 (L) = using Leica reagents/platforms, 30-9 (UV) = 30-9 using UltraView detection (Ventana), 30-9 (OV) = 30-9 using OptiView detection (Ventana), MM1 (L) = MM1 using Leica reagents/platforms, MM1 (nL) = MM1 using non-Leica reagents/platforms, SP6 (UV) = SP6 using UltraView detection (Ventana)

	All [1]	30-9 (UV)	30-9 (OV)	K2 (All)	MIB1 (D)	MIB1 (L)	MIB1 (V)	SP6 (UV)	MM1 (All L)	MM1 (nL)
Number of values	2466	232	173	142	335	381	488	48	182	72
25% Percentile	14	16	16	16	15	11	14	15	13	11
Median	**16**	**17**	**17**	**16**	**16**	**14**	**16**	**16**	**16**	**14**
75% Percentile	17	18	18	17	17	16	17	17	16	16
Mean	**15.3**	**17.2**	**16.8**	**16.3**	**16.0**	**13.3**	**15.5**	**15.7**	**14.5**	**13.3**
Std. Deviation	2.8	1.7	2.1	2.1	2.4	3.0	2.8	1.9	2.8	3.2
S.E. Mean	0.06	0.11	0.16	0.18	0.13	0.15	0.13	0.28	0.21	0.38
Lower 95% CI mean	15.2	17.0	16.4	15.9	15.8	13.0	15.3	15.1	14.1	12.6
Upper 95% CI mean	15.4	17.4	17.1	16.6	16.3	13.6	15.8	16.2	14.9	14.1

Table 2. Overall and optimised primary antibody performance. Showing the data illustrated in Figure 3 and additional descriptive statistics. Key is same as for Figure 2.

Take home messages:

- In this study, **MIB1** was the most widely used primary antibody clone,
- Performance of primary antibodies varied considerably,
- **30-9** was associated with the best scores, **MM1** with the worst,
- Methodology parameters affected primary antibody performance significantly – evidence for this was seen for **MIB1** and **MM1** in particular.

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