Oestrogen receptor antibodies and their performance in the UK National External Quality Assessment Scheme for Immunocytochemistry and In-situ Hybridisation (UK NEQAS ICC & ISH)

UK NEQAS
International Quality Expertise

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BACKGROUND

UK NEQAS ICC & ISH (www.ukneqasiccish.org) is one of the world's largest proficiency testing organisations, both in terms of scope and participant numbers.

Established in London in 1985, UK NEQAS ICC & ISH has developed into an international scheme serving laboratories in the UK and across the world. It provides quality assessment of immunocytochemistry (ICC) and *in-situ* hybridisation (ISH) used in the demonstration of a large range of clinically important markers.

The Scheme's Breast Steroid Hormone Module assesses proficiency in the demonstration of oestrogen receptor (ER) and progesterone receptor (PR) within the context of breast cancer predictive testing.

We have examined data gathered in the course of ER assessments conducted over a period spanning more than 10 years (2007-2018).

MATERIALS and METHODS

Three assessments of staining for ER are conducted per year. At each assessment, participants are provided with unstained sections from formalin fixed, paraffin embedded breast cancer samples known to express ER at high and moderate levels respectively, together with sections from an ER-negative tumour. They are required to stain these together with their own in-house control tissue(s), using their standard methodologies. Stained sections are returned and assessed for correct demonstration of expected ER expression levels (UK NEQAS provided materials), and suitable choice of ER-expression range (in-house materials), and for other aspects of technical quality.

UK NEQAS and in-house materials are assessed separately. In both cases, assessment is carried out by four expert assessors working independently to a pre-specified set of standards. Dependent on overall quality, they award a mark in the range 1–5, the four assessors marks are summed to give the participant's final score in the range 4–20 (see Table 1).

Data were collated and examined on primary antibody clone and supplier and automated immuno-staining platform supplier for assessment runs that happened between 2007 and early-2018.

MATERIALS and METHODS (continued)

Descriptive statistics are presented for primary antibody clone use, proportion of participants achieving acceptable staining by clone and use of immuno-staining platform by supplier. Data were curated and analysed in Excel (Microsoft).

Assessor's Mark	Quality Descriptor (UK NEQAS materials)
1	Unacceptable. No reliable information can be obtained.
2	Unacceptable. Clinically incorrect staining-level(s) in at least one tumour.
3	Borderline. Clinically appropriate staining-levels, but significant technical improvement(s) required.
4	Acceptable. Clinically appropriate staining-levels. Minor technical issue(s) present.
5	Optimal staining.
Final Score	**Final Assessed Category**
4 to 9	Unacceptable (fail).
10 to 12	Borderline (pass).
13 to 20	Acceptable (pass).

TABLE 1. Scoring guidelines for ER assessments.

RESULTS

Assessment runs included in this analysis occurred in the period between Quarter 3 (Q3), 2007 and Quarter 1 (Q1), 2018 (N = 34). The median number of participating laboratories per run was 333 (range: 264–368). In the course of the examined period a total of 546 different laboratories participated. Total number of submissions was 10,902. Of these, 5,407 (49.6%) were from laboratories located in the UK, and 5,495 (50.4%) from laboratories outside the UK (60 non-UK countries represented). One of five primary antibody clones (supplied in the majority of cases by four companies) was used for the demonstration of ER in 91.8% of cases (see Table 2). No other ER primary antibody was used for a total of >0.2% of submissions. Trends over time with regard to proportions of submissions for each antibody clone were clearly seen; in particular there was a proportional reduction in 1D5 and 6F11 use and an increase in the numbers of participants using SP1 and EP1, see Chart 1.

Primary Antibody Clone	Total Participations		Antibody Principle Supplier		
			Name	Market Share	
	N	%		N	%
1D5	736	*6.8*	Agilent Dako	716	*97.3*
6F11	4955	*45.5*	Leica	3733	*75.3*
EP1	772	*7.1*	Agilent Dako	772	*100.0*
SP1	3549	*32.6*	Ventana	2881	*81.2*
Other/NS	890	*8.2*	Various/NS	n/a	*n/a*
Total	**10902**	***100.0***			

TABLE 2. ER primary antibody use and supplier details. (NS = Not Specified, n/a = not applicable)

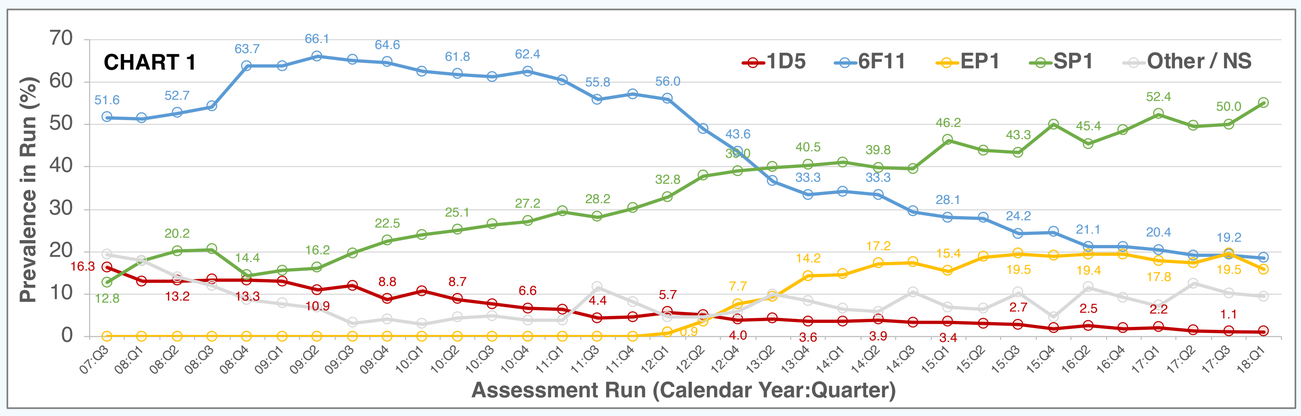

CHART 1. Trends in ER primary antibody use over time.
Showing change in the proportion of participants using each of the commonly employed clones over duration of the study period. Data labels indicate proportion in run (alternate run data points shown for clarity, and not shown at all for Other/NS category). Note that clone EP1 was introduced towards the end of 2011. NS = Not Specified

CHART 2A-C: Final score achieved on UK NEQAS supplied samples used to categorise results as a Pass (scores 10–20) or a Fail (scores 4–9). Bold lines indicate moving average over 5 assessment runs.
Proportion of participants achieving a Pass at each assessment run was calculated for the four main primary antibody clones. This was done separately for the whole data-set, for non-UK participants and for UK-resident participants (Charts 2A-C, respectively). Similar outcomes were seen within all three analysed data-sets; the users of the two rabbit monoclonal clones (EP1 and SP1) more frequently achieved a Pass at assessment compared to participants using the mouse monoclonals (1D5 and 6F11). Table 3 presents summary data for the same three data-sets, and shows the assessment outcome 'Pass' further divided into Borderline and Acceptable.

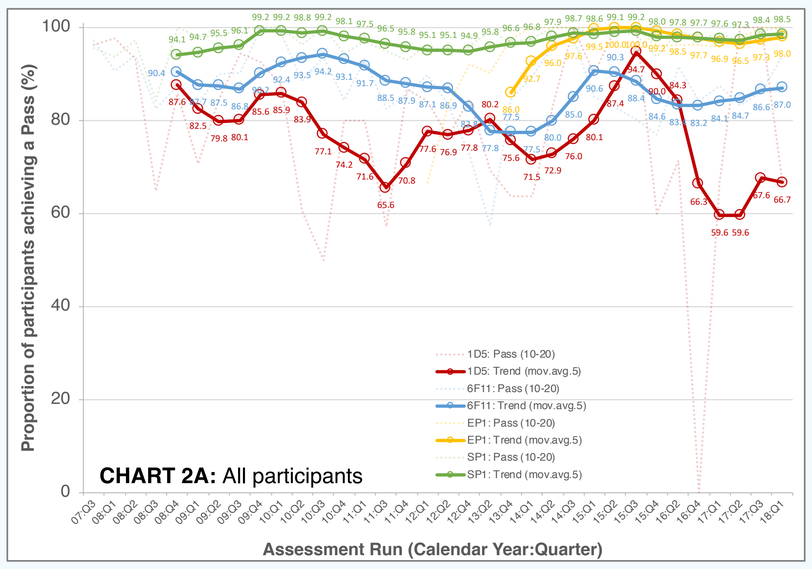

CHART 2A: All participants

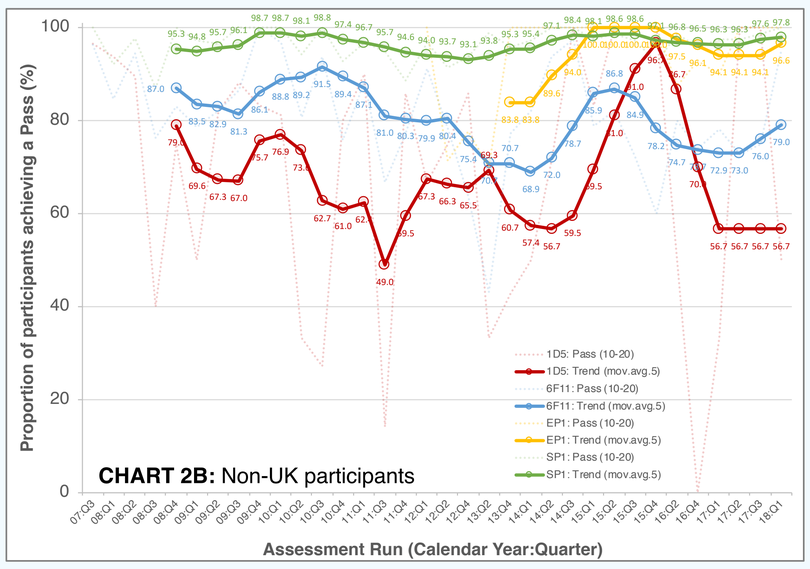

CHART 2B: Non-UK participants

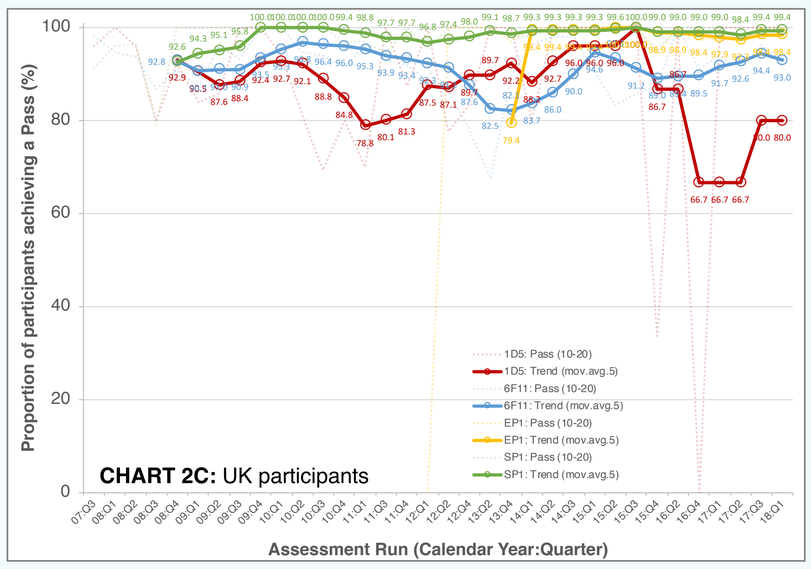

CHART 2C: UK participants

Primary Antibody Clone	All		Non-UK		UK	
	N	%	N	%	N	%
1D5						
Unacceptable: Fail (4-9)	126	*18.5*	87	*28.1*	39	*10.5*
Borderline: Pass (10-12)	188	*27.6*	96	*31.0*	92	*24.9*
Acceptable: Pass (13-20)	366	*53.8*	127	*41.0*	239	*64.6*
Overall Pass (10-20)	**554**	**81.5**	**223**	**71.9**	**331**	**89.5**
6F11						
Unacceptable: Fail (4-9)	582	*11.9*	358	*17.2*	224	*8.0*
Borderline: Pass (10-12)	962	*19.7*	472	*22.6*	490	*17.6*
Acceptable: Pass (13-20)	3329	*68.3*	1254	*60.2*	2075	*74.4*
Overall Pass (10-20)	**4291**	**88.1**	**1726**	**82.8**	**2565**	**92.0**
EP1						
Unacceptable: Fail (4-9)	18	*2.3*	11	*5.5*	7	*1.2*
Borderline: Pass (10-12)	62	*8.0*	21	*10.6*	41	*7.2*
Acceptable: Pass (13-20)	691	*89.6*	167	*83.9*	524	*91.6*
Overall Pass (10-20)	**753**	**97.7**	**188**	**94.5**	**565**	**98.8**
SP1						
Unacceptable: Fail (4-9)	92	*2.6*	70	*3.4*	22	*1.5*
Borderline: Pass (10-12)	331	*9.5*	202	*9.9*	129	*8.9*
Acceptable: Pass (13-20)	3064	*87.9*	1768	*86.7*	1296	*89.6*
Overall Pass (10-20)	**3395**	**97.4**	**1970**	**96.6**	**1425**	**98.5**

TABLE 3. Overall outcome across all assessment runs for each ER primary antibody clone.
Overall pass rate for each is indicated using bold/shading.

Platform Supplier	All		Non-UK		UK	
	N	%	N	%	N	%
Agilent Dako	2141	*19.6*	834	*15.2*	1307	*24.2*
Leica Biosystems	2576	*23.6*	1041	*18.9*	1535	*28.4*
Other/NS	1638	*15.0*	952	*17.3*	686	*12.7*
Ventana Medical Systems	4280	*39.3*	2456	*44.7*	1824	*33.7*
Manual	267	*2.4*	212	*3.9*	55	*1.0*
Totals	10902	*100.0*	5495	*100.0*	5407	*100.0*

TABLE 4. Frequency for use of automated immuno-staining platforms grouped by supplier and for use of non-automated staining (manual).
Suppliers of platforms used by <4% of participants are grouped into 'Other/NS'. NS = Not Specified

Platform Supplier	1D5			6F11			EP1			SP1			Totals		
	N	%	Mean Score	N	%	Mean Score	N	%	Mean Score	N	%	Mean Score	N	%	Mean Score
Agilent Dako	417	*21.0*	**12.5**	917	*46.1*	**13.8**	377	*19.0*	**16.1**	278	*14.0*	**14.3**	1989	*100*	**14.0**
Leica Biosystems	77	*3.1*	**13.1**	1964	*78.6*	**13.8**	325	*13.0*	**15.6**	134	*5.4*	**15.2**	2500	*100*	**14.0**
Ventana Medical Systems	6	*0.1*	**6.2**	1109	*27.2*	**14.0**	26	*0.6*	**15.8**	2942	*72.1*	**15.5**	4083	*100*	**15.1**
Manual	45	*19.8*	**8.7**	150	*66.1*	**13.1**	16	*7.0*	**14.3**	16	*7.0*	**8.9**	227	*100*	**12.0**
Totals	545	*6.2*	**12.2**	4140	*47.1*	**13.8**	744	*8.5*	**15.8**	3370	*38.3*	**15.4**	8799	*100*	**14.5**

TABLE 5. Distribution of primary antibody clone use on each supplier's automation (and manual), together with information about the outcome (mean assessment score).
Key to Mean Score colour coding: orange = Unacceptable/Fail (4-9); blue = Borderline/Pass (10-12); pale yellow = Acceptable/Pass (13-15); green = Acceptable/Pass (16-20).

CONCLUSIONS

The last decade has seen a significant change in the 'landscape' of ER primary antibody use. There has been increasing use of rabbit monoclonal reagents (SP1 and EP1) and a concomitant decrease in the use of their mouse monoclonal counterparts (1D5 and 6F11). Superior results at EQA, which are largely platform independent reflect the reasons underlying these changes.

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