The authors declare no financial disclosure or conflict of interest that relate to the research presented in this poster

USCAP 114th Annual Meeting, March 22-27, 2025, Boston, Massachusetts

UK NEQAS Immunocytochemistry & In-Situ Hybridisation

Application of Digital Image Analysis for the Assessment of HER2-low in Breast Cancer – Comparison with Visual Assessments Performed at The UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation (UK NEQAS ICC & ISH) HER2-low EQA Programme

Lila Zabaglo [1], Suzanne Parry [1], Dawn Wilkinson [1], Andrew Dodson [1]
[1] UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation, London UK. Corresponding author's email address: adodson@ukneqasiccish.org

Background and Aims

- Accurate assessment of lower ranges of HER2 immunohistochemical (IHC) expression has become important since trastuzumab deruxtecan (T-DXd) was approved for treatment of patients with HER2-low metastatic breast cancer (BC) [Ref.1].
- Findings from DESTINY-Breast06 Phase 3 trial showed that T-DXd offers a favourable outcome over standard chemotherapy in patients with HER2-low and HER2-ultra-low metastatic BC [Ref.2].
- Several studies showed low concordance among pathologists in distinguishing low levels of HER2 IHC expression.
- There is an emerging need for a standardized and reproducible HER2-low testing methodology.
- A dedicated EQA programme for the assessment of IHC stain quality of HER2-low testing was established by UK NEQAS ICC & ISH in 2023 providing support to clinical laboratories and gathering evidence about the reproducibility of HER2-low testing.
- We report results from the application of digital image analysis (DIA) for the HER2-low IHC assessment with a view of implementing DIA in the HER2-low EQA programme.

Materials and Methods

- Formalin-fixed paraffin embedded (FFPE) sections from a set of BC specimens showing a range of HER2 expression (from 0 to 2+) were prepared onto microscope slides (Fig. 1).
- Unstained sections were distributed to laboratories participating in the UK NEQAS ICC & ISH HER2-Low EQA programme.
- The participating laboratories stained the slides using their routine IHC methods for HER2 and returned for central assessment by an expert panel of assessors.
- Slides were scanned at x40 using a NanoZoomer (Hamamatsu, Japan).
- Digital image analysis (DIA) using Visiopharm HER2 APP 10185 (Visiopharm A/S, Hørsholm, Denmark) was applied to:
  - training set of 272 samples that were selected and scored by four Assessors as confident HER2 0, 1+, and 2+ samples.
  - 1554 samples from four EQA Runs (Runs 144-147 conducted at 4-monthly intervals).
- Agreement between DIA and visual scores was assessed using Spearman correlation coefficient.

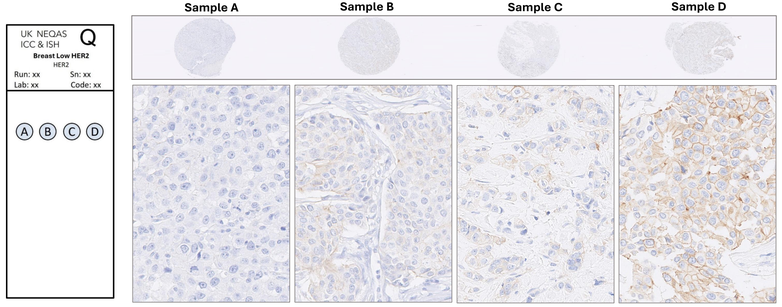

Fig. 1. UK NEQAS ICC & ISH HER2-Low EQA programme slide layout. TMA cores taken from FFPE breast cancer excision tissues (samples A, B, C, and D) showing varying levels of HER2 membrane protein expression (from HER2 negative to HER2 2+) were provided as the assessment samples.

HER2 Assessment

Visual assessment was performed by four Assessors experienced in breast HER2 IHC evaluation who scored the preparations concurrently around a multi-header microscope. Each Assessor rated the HER2 category. Scores which disagreed were resolved by consensus agreement.

DIA assessment was conducted using Visiopharm HER2 APP 10185 (Fig.2). It is an automatic single-cell approach APP for HER2 IHC quantification using an algorithm aligned with ASCO/CAP HER2 IHC guidelines for 0, 1+, 2+, and 3+ categorization.

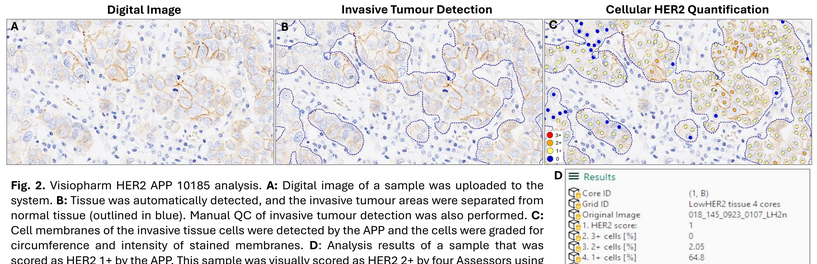

Fig. 2. Visiopharm HER2 APP 10185 analysis. **A:** Digital image of a sample was uploaded to the system. **B:** Tissue was automatically detected, and the invasive tumour areas were separated from normal tissue (outlined in blue). Manual QC of invasive tumour detection was also performed. **C:** Cell membranes of the invasive tissue cells were detected by the APP and the cells were graded for circumference and intensity of stained membranes. **D:** Analysis results of a sample that was scored as HER2 1+ by the APP. This sample was visually scored as HER2 2+ by four Assessors using the UK Recommendations for HER2 Assessment highlighting the differences between the UK and ASCO/CAP guidelines.

Results

Training set: The correlation between DIA and visual scores was r = 0.91 (p<0.0001), (Fig.3A). In the training set:

- 93% specimens assessed visually as 'HER2 0' and 99% specimens visually scored as 'HER2 1+' were DIA scored as '0' and '1+' respectively.
- 31% of samples visually scored as '2+' were also scored as '2+' by DIA, while the remaining BCs were scored as '1+'.

EQA Runs 144-147: Slightly lower correlation was observed in BC samples (n= 1554) from four EQA Runs (r = 0.83, p<0.0001), (Fig.3B). In this set:

- 91% of samples visually classified as '0' and 92% specimens visually scored as '1+' were DIA scored as '0' and '1+' respectively (Fig.4).
- Similarly to the training set, lower percentage (34.6%) of samples that were scored visually as '2+' were DIA classified as '2+', while the majority of remaining BCs were DIA scored as '1+'. There were two cores (<1%) that were DIA scored as '3+', (Fig.4).

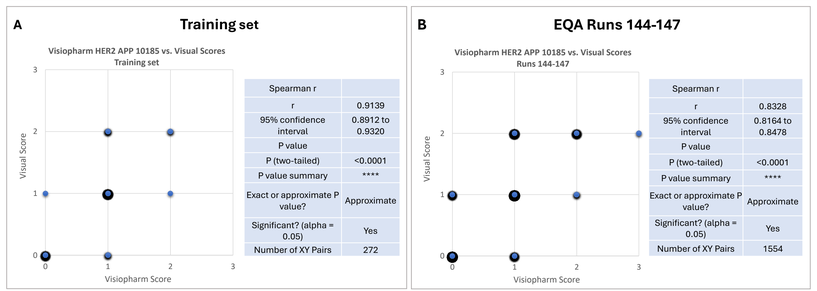

Fig. 3. Correlation between Visiopharm DIA and Assessors' visual scores in: (**A**) training set; (**B**) BC samples from four EQA runs. Agreement between DIA and visual scores was assessed using Spearman correlation coefficient.

Results

RUN 144			RUN 145			RUN 146			RUN 147			ALL RUNS 144-147		
Run 144 All cores			Run 145 All cores			Run 146 All cores			Run 147 All cores			Runs 144-147 ALL Cores		
Total	369	%	Total	390	%	Total	373	%	Total	422	%	Total	1554	%
Matched	270	73.17	Matched	329	84.36	Matched	319	85.52	Matched	380	90.05	Matched	1298	83.53
Mismatched:	99	26.83	Mismatched:	61	15.64	Mismatched:	54	14.48	Mismatched:	42	9.95	Mismatched:	256	16.47
DIA Upgraded from 0 to 1+	25	6.78	DIA Upgraded from 0 to 1+	5	1.28	DIA Upgraded from 0 to 1+	12	3.22	DIA Upgraded from 0 to 1+	11	2.61	DIA Upgraded from 0 to 1+	53	3.41
DIA Upgraded from 2+ to 3+	2	0.54				DIA Upgraded from 1+ to 2+	1	0.27	DIA Upgraded from 1+ to 2+	5	1.18	DIA Upgraded from 1+ to 2+	6	0.39
DIA Downgraded from 1+ to 0	6	1.62	DIA Downgraded from 1+ to 0	16	4.10	DIA Downgraded from 1+ to 0	6	1.61	DIA Downgraded from 2+ to 3+			DIA Upgraded from 2+ to 3+	2	0.13
DIA Downgraded from 2+ to 1+	66	17.89	DIA Downgraded from 2+ to 1+	40	10.26	DIA Downgraded from 2+ to 1+	35	9.38	DIA Downgraded from 2+ to 1+	1	0.24	DIA Downgraded from 1+ to 0	53	3.40
												DIA Downgraded from 2+ to 1+	142	9.14
Run 144 Cores Visually scored '0'			Run 145 Cores Visually scored '0'			Run 146 Cores Visually scored '0'			Run 147 Cores Visually scored '0'			Runs 144 -147 Cores Visually scored '0'		
Total	136	%	Total	149	%	Total	133	%	Total	151	%	Total	569	%
Matched	111	81.62	Matched	144	96.64	Matched	121	90.98	Matched	140	92.72	Matched	516	90.69
Mismatched:	25	18.38	Mismatched:	5	3.36	Mismatched:	12	9.02	Mismatched:	11	7.28	Mismatched:	53	9.31
DIA Upgraded from 0 to 1+	25	18.38	DIA Upgraded from 0 to 1+	5	3.36	DIA Upgraded from 0 to 1+	12	9.02	DIA Upgraded from 0 to 1+	11	7.28	DIA Upgraded from 0 to 1+	53	9.31
Run 144 Cores Visually scored '1+'			Run 145 Cores Visually scored '1+'			Run 146 Cores Visually scored '1+'			Run 147 Cores Visually scored '1+'			Runs 144 -147 Cores Visually scored '1+'		
Total	114	%	Total	193	%	Total	197	%	Total	261	%	Total	765	%
Matched	108	94.74	Matched	177	91.71	Matched	190	96.45	Matched	231	88.51	Matched	706	92.29
Mismatched:	6	5.26	Mismatched:	16	8.29	Mismatched:	7	3.55	Mismatched:	30	11.49	Mismatched:	59	7.71
DIA Downgraded from 1+ to 0	6	5.26	DIA Downgraded from 1+ to 0	16	8.29	DIA Downgraded from 1+ to 0	6	3.05	DIA Downgraded from 1+ to 0	25	9.58	DIA Downgraded from 1+ to 0	53	6.93
						DIA Upgraded from 1+ to 2+	1	0.50	DIA Upgraded from 1+ to 2+	5	1.91	DIA Upgraded from 1+ to 2+	6	0.78
Run 144 Cores Visually scored '2+'			Run 145 Cores Visually scored '2+'			Run 146 Cores Visually scored '2+'			Run 147 Cores Visually scored '2+'			Runs 144-147 Cores Visually scored '2+'		
Total	119	%	Total	48	%	Total	43	%	Total	10	%	Total	220	%
Matched	51	42.86	Matched	8	16.67	Matched	8	18.60	Matched	9	90.00	Matched	76	34.55
Mismatched:	68	57.14	Mismatched:	40	83.33	Mismatched:	35	81.40	Mismatched:	1	10.00	Mismatched:	144	65.45
DIA Downgraded from 2+ to1+	66	55.46	DIA Downgraded from 2+ to1+	40	83.33	DIA Downgraded from 2+ to 1+	35	81.40	DIA Downgraded from 2+ to 1+	1	10.00	DIA Downgraded from 2+ to 1+	142	64.55
DIA Upgraded from 2+ to 3+	2	1.68										DIA Upgraded from 2+ to 3+	2	0.90

Fig. 4. Comparison of Assessors' visual and Visiopharm DIA scores in individual EQA Runs and in BC samples pooled form four runs. Numbers of matched and mismatched cases together with percentages are shown.

Conclusions

- Overall, strong agreement between visual and DIA scores was observed for both HER2 0 and HER2 1+ BCs in both training and EQA Runs 144-147 data sets. A significant level of disagreement was observed in the cases assigned as 2+ by visual assessment, where more than two-thirds of which were scored as 1+ by DIA.
- In samples from four EQA Runs:
  - DIA scored 53 cases (9.3%) as 1+, which visually were classified as negative. In 40% of cases DIA classified as HER2 1+, staining was close to the 10% threshold currently established by UK and ASCO/CAP guidelines.
  - The membrane staining of 53 cases (6.9%) was downgraded by DIA from HER2 1+ to 0. In approximately 50% of these cases, the number of cells with HER2 1+ staining was close to the 10% threshold.
  - Much larger variability was observed for BCs visually assessed as '2+' where only 34.6% of samples were DIA classified as '2+', while majority of BCs were DIA scored as '1+'. The results may mirror the overscoring of HER2 2+ tumours that is currently observed in the UK diagnostic community using the UK guidelines.
- DIA enhanced the HER2 scoring reproducibility and precision particularly in cases scoring close to categorical cut-off points.
- Implementing DIA in the HER2-low EQA programme will also improve workflow efficiency.

Acknowledgements

Authors gratefully acknowledge assistance of Visiopharm and contribution of Assessors participating in the EQA assessments.

References

Ref.1 Narayan, P., et al., Clin Cancer Res, 2021. 27(16): 4478-4485
Ref.2 Bardia, A., et al., N Engl J Med, 2024. 391(22): 2110-2122