

Use of digital image analysis in UK NEQAS for Immunocytochemistry and In-Situ Hybridization Assessments

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Lay Summary: The UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation (UK NEQAS ICC & ISH) assesses the technical quality of immunocytochemistry (ICC). There are increasing requirements for quantitative scoring of ICC, particularly of biomarkers, and digital image analysis (DIA) systems are being introduced. We examined the efficacy of DIA using Cognition Master for breast cancer samples stained for the estrogen receptor (ER), and demonstrate the potential use of DIA to support assessments.

AIMS: To examine the feasibility of using DIA to provide additional information in support of traditional, visual microscopic viewing of participant materials.

MATERIALS AND METHODS: Tissue microarray (TMA) samples comprising of tonsil, ER-negative, ER-mid, and ER-high expressing tumours were stained for estrogen receptor (ER)-immunoreactivity, either as UK NEQAS Reference Slides or by participants to the EQA scheme. UK NEQAS Reference Slides were scanned at x20 magnification using a NanoZoomer S210 (Hamamatsu) digital slide scanner.

NPD image viewing software (Hamamatsu) was used to export whole TMA cores and regions of interest (ROI) as a JPEG image files. DIA of both whole cores and ROI was performed using the ER Quantifier module of Cognition Master (VMscope).

Participant EQA slides were first visually assessed by a panel of four assessors prior to DIA (n=38), prior to regional Cognition Master analysis.

RESULTS: Full Core Digital Image Analysis

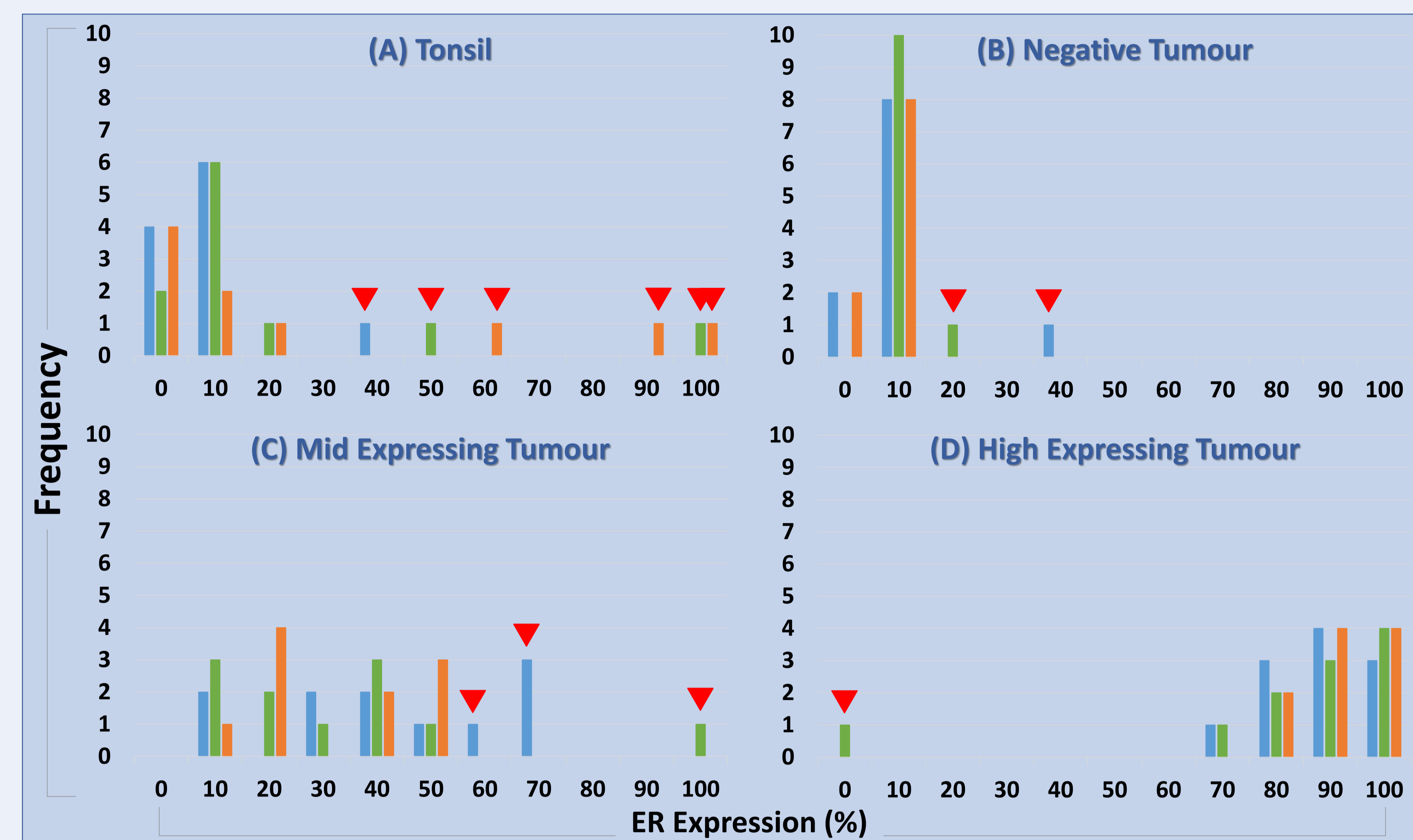


Figure 1: Full core DIA. Whole tonsil (A), Negative (B), Mid- (C), and High-expressing (D) TMA cores were scanned three times and DIA performed.

Whole TMA cores from UK NEQAS Reference Slides were analysed by DIA since it was hypothesized that whole core analysis may reduce variability and increase accuracy. Moreover, the same TMA cores were scanned and analysed three times to assess the reproducibility of data.

Based on the average of all three scans, ER expression was:

- Detected in >60% of the high-expressing tumour cells by 96.9% of participants (Median expression: 86.1%, inter quartile range (IQR): 11.87%). See Figure 1D.
- In the mid-expressing tumours, most (84.4%) scans had between ≤10% and 50% ER expression (Median expression: 28.7%, inter quartile range (IQR): 27.8%). See Figure 1C.
- The majority of scans had ≤10% ER expression in the Negative expressing tumour (93.5%) and in the tonsil (75.0%). See Figure 1A and 1B.

	Tonsil: Mean	Tonsil: Median	Tonsil: IQR	Neg: Mean	Neg: Median	Neg: IQR	Mid: Mean	Mid: Median	Mid: IQR	High: Mean	High: Median	High: IQR
Original Scans	4.2	0.1	1.2	3.4	0.0	0.1	38.2	35.9	32.5	83.1	84.2	15.9
Re-Scanned	14.5	1.1	6.7	1.5	0.1	0.2	29.0	24.0	26.8	76.5	87.3	12.4
Re-Scan 2	25.4	0.3	46.4	0.1	0.0	0.0	26.5	26.2	24.3	87.1	89.4	7.3
Mean	14.7	0.5	18.1	1.6	0.1	0.1	31.2	28.7	27.8	82.2	87.0	11.9

Legend: Original Scans (blue), Re-Scanned (green), Re-Scanned 2 (orange), Outlier (red triangle)

Region of Interest Digital Image Analysis:

Since whole core analysis resulted in a number of outliers and inconsistent data across the independent scans, regional analysis of the TMAs was performed. Three ROI were analysed per core by DIA from UK NEQAS Reference Slides. The same TMA cores were scanned and analysed three times to assess the reproducibility of data.

Based on the average of all three scans, ER expression was:

- Detected in ≥60% of the high-expressing tumour cells by 90.0% of participants (Median expression: 66.7%, inter quartile range (IQR): 9.3%). See Figure 2D.
- In the mid-expressing tumours, most (96.8%) scans had between 20% and 60% ER expression (Median expression: 47.2%, IQR: 20.6%). See Figure 2C.
- The majority of scans had ≤10% ER expression in the Negative expressing tumour (100%) and in the tonsil (96.9%). See Figure 2A and 2B.

	Tonsil: Mean	Tonsil: Median	Tonsil: IQR	Neg: Mean	Neg: Median	Neg: IQR	Mid: Mean	Mid: Median	Mid: IQR	High: Mean	High: Median	High: IQR
Original Scans	0.9	0.1	0.0	0.6	0.6	2.9	0.0	0.0	0.6	0.6	43.8	50.7
Re-Scanned	0.6	0.1	0.0	0.4	0.4	0.4	0.0	0.0	0.2	0.2	39.9	45.0
Re-Scan 2	0.8	0.3	0.0	1.0	1.0	0.3	0.0	0.0	0.1	0.1	42.3	45.9
Mean	0.8	0.1	0.0	0.7	0.7	1.2	0.0	0.0	0.3	0.3	42.0	47.2

Legend: Original Scans (blue), Re-Scanned (green), Re-Scanned 2 (orange), Outlier (red triangle)

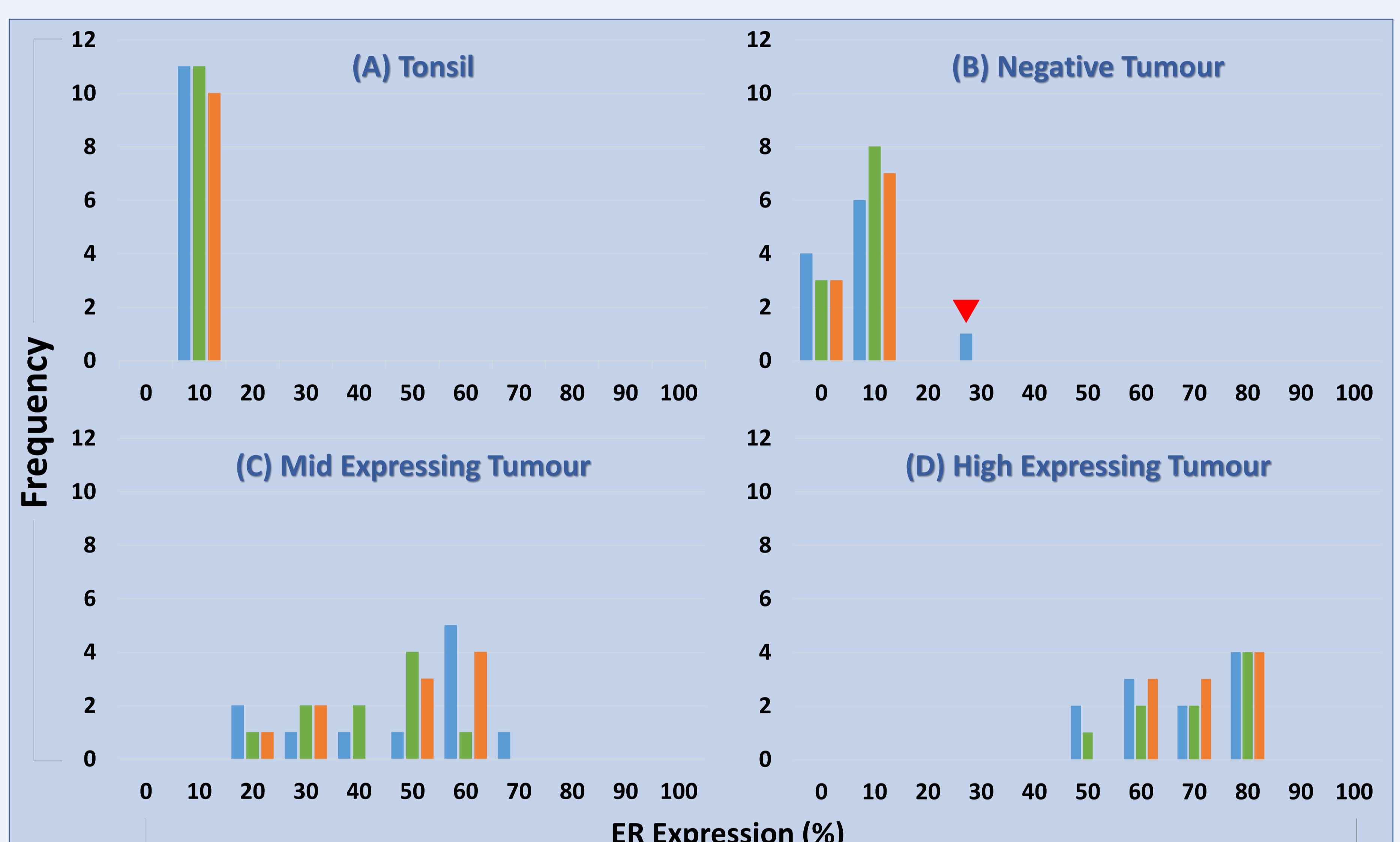


Figure 2: Region of Interest DIA. ROI for tonsil (A), Negative (B), Mid- (C), and High-expressing (D) TMA cores were scanned three times and DIA performed.

Application of Regional Analysis: Since ROI analysis improved the accuracy and reproducibility of data in comparison to whole core analysis, regional analysis was applied to participant EQA slides (n=39).

	Tonsil: Mean	Tonsil: Median	Tonsil: IQR	Neg: Mean	Neg: Median	Neg: IQR	Mid: Mean	Mid: Median	Mid: IQR	High: Mean	High: Median	High: IQR
Original Scans	0.9	0.1	0.0	0.6	0.6	2.9	0.0	0.0	0.6	0.6	43.8	50.7
Re-Scanned	0.6	0.1	0.0	0.4	0.4	0.4	0.0	0.0	0.2	0.2	39.9	45.0
Re-Scan 2	0.8	0.3	0.0	1.0	1.0	0.3	0.0	0.0	0.1	0.1	42.3	45.9
Mean	0.8	0.1	0.0	0.7	0.7	1.2	0.0	0.0	0.3	0.3	42.0	47.2

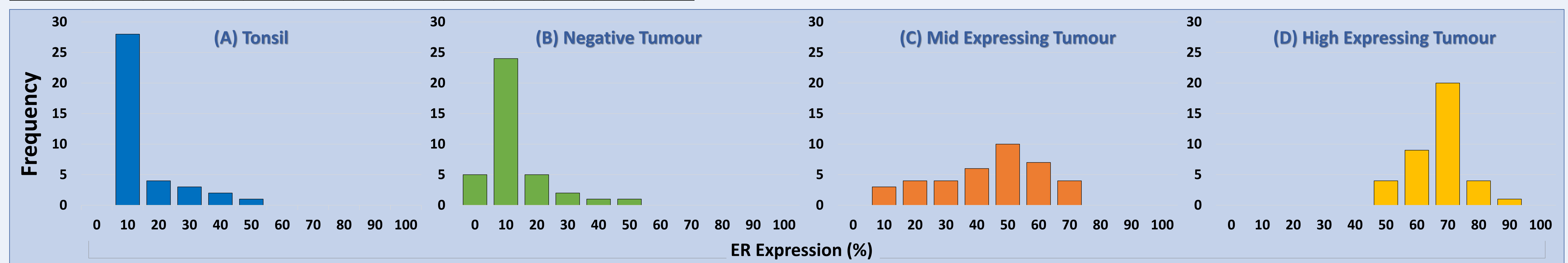


Figure 3: Regional Analysis of participant EQA slides

Take home messages:

- Regional DIA of ER-expression improved the accuracy and reproducibility of data compared to whole core analysis.
- Mid-expressing samples exhibited the greatest variability in the technical quality of staining by visual assessment, and the broadest spread in the percentage of ER-positive tumour cells by Cognition Master analysis.
- We demonstrate a concordance between the quantitative data obtained here and the quality of immunostaining.
- Regional analysis of ER-expression using Cognition Master demonstrates the potential use of DIA to support UK NEQAS EQA assessments.