External Quality Assessment for PD-L1 Testing in Triple Negative Breast Cancer

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BACKGROUND

PD-1/PD-L1 inhibitors have become first line treatment options for patients with advanced disease in many tumour types. They are effective in a subset of patients with advanced triple negative breast cancer (TNBC). Immunocytochemistry assays are used to predict which patients may benefit, and for each indication specific companion assays are recommended.

The UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation (UK NEQAS ICC & ISH) assesses technical quality of laboratory testing.

Here we report on a pilot EQA for PD-L1 staining in TNBC and present results from the first assessment.

MATERIALS and METHODS

Unstained composite slides consisting of FFPE tonsil, TNBC tissue samples, and cell lines of known PD-L1 expression levels were distributed. Participants stained these slides for PD-L1 using their routine method and returned them for assessment.

Reference slides at intervals of every 25 sections were stained for H&E and for PD-L1 using the SP142 assay (Ventana 741-4860). Stained reference and participant slides were scanned at x20 using a NanoZoomer S210 (Hamamatsu).

Assessors reviewed the digitised reference slides and established the tumour-associated immune scores (IC) for the breast cancers, and the tumour percentage scores (TPS) for the cell lines (Figure 1).

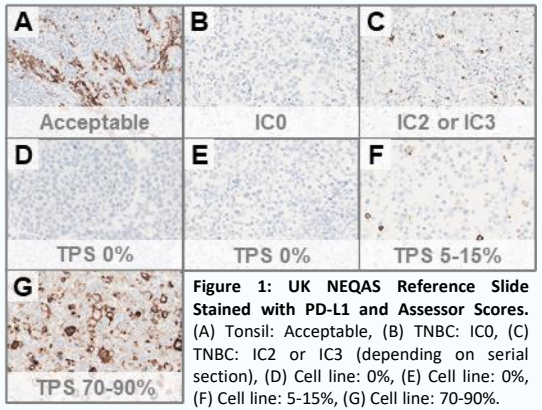


Figure 1: UK NEQAS Reference Slide Stained with PD-L1 and Assessor Scores. (A) Tonsil: Acceptable, (B) TNBC: IC0, (C) TNBC: IC2 or IC3 (depending on serial section), (D) Cell line: 0%, (E) Cell line: 0%, (F) Cell line: 5-15%, (G) Cell line: 70-90%.

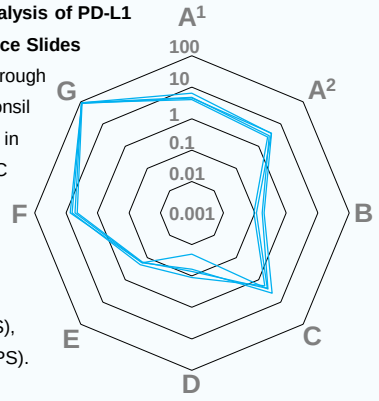
Participant slides were assessed by the same panel of four assessors and awarded a total mark in the range of 4-20 (**≤8** Unacceptable – FAIL, **12** Borderline Acceptable – PASS, **13-15** Acceptable – PASS, **16-20** Excellent – PASS).

Image Analysis: Three different VisioPharm APPs were trained on the reference samples to quantify PD-L1 expression in the tonsil, TNBC, and cell line samples, respectively. These were used to analyse the participant slides (see spider Plots 1 and 2).

RESULTS

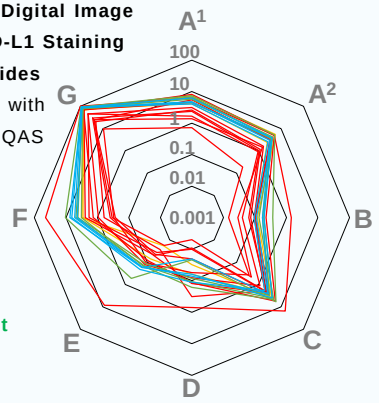
Overall Participant Scores: A total of 16 participant submissions were received. 10 laboratories (62.5%) failed with scores ≤8, 2 laboratories (12.5%) passed with a borderline acceptable score of 12, no laboratories fell into the acceptable category with scores of 13-15, and 4 laboratories (25%) received an excellent pass with scores of 16-20.

Plot 1: Quantitative Analysis of PD-L1 Staining in the Reference Slides showing little variation through the samples. (A^1) % in tonsil germinal centres, (A^2) % in whole tonsil, (B) % TNBC tumour region, (C) % TNBC tumour region, (D) Cell line (<1% TPS), (E) Cell line (<1% TPS), (F) Cell line (5-15% TPS), (G) Cell line (70-90% TPS).



Plot 2: Quantitative Digital Image Analysis (DIA) of PD-L1 Staining in the Participant Slides showing concordance with the qualitative UK NEQAS scores in the tonsil, TNBC and cell line samples.

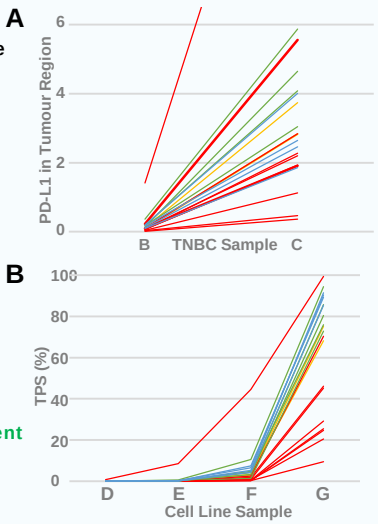
Reference, **Fail**, **Borderline**, **Excellent**



Participant Staining and DIA of TNBC and Cell Line Samples: Only 25% of participants achieved expected levels of staining on cell line samples, whereas 87.5% were able to do so on the breast cancers (see Plot 3). There was 100% agreement between manual and DIA scoring in all cases. The majority (87.5%) of participants were using the Ventana SP142 assay (FDA and EU approved), but many were using it in protocols that differed from the approved one e.g. shorter or longer antigen retrieval, or without amplification, leading to very poor reproducibility amongst the group.

Plot 3. Quantitative DIA for TNBC (A) and Cell Line (B) Samples showing staining by each laboratory. Weak staining of PD-L1 was observed by the majority of laboratories who failed.

Reference, **Fail**, **Borderline**, **Excellent**



CONCLUSIONS

We present results from the first UK NEQAS ICC & ISH EQA assessment for PD-L1 in TNBC.

Use of the SP142 assay in non-approved IHC protocols leads to a wide variation of results.

Manual EQA assessment of slides was supported by concordant quantative DIA of PDL1 staining.

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