Cell Line Control Update by UKNEQAS ICC & ISH and Leica Biosystems, Newcastle

Part 2 – Cell Line Quality Control

Merodl Ibrahim & Keith Miller, UKNEQAS, Immunocytochemistry and In Situ Hybridisation
Michael Gandy, Craig Barker & Vicky Reid, Leica Biosystems Ltd, Newcastle upon Tyne, UK

Introduction

- At Leica Biosystems all control cell lines undergo strict quality control evaluations using Haematoxylin & Eosin (H&E) and IHC stained sections. This allows for evaluation of the three main cell line characteristics: Cellular Morphology, IHC Profile and Core Density.
- The evaluation of control cell lines should always be performed within the context of appropriate tolerance levels as subtle changes in both protein and gene expression from batch-to-batch may occur. Screening out cells that fall outside these tolerance criteria is part of the QC process. This enables the correct evaluation of cell line staining patterns within appropriate tolerance levels, allowing control cell lines to be utilized both as commercial system and EQA monitoring devices.
- The aims of this update are to:
  - Outline the key quality control procedures that are implemented, to ensure that the control cell lines utilized by UKNEQAS ICC & ISH in the Breast HER2 IHC and ISH modules maintain the highest standards of consistency and reproducibility.
  - Highlight the expected staining patterns and interpretation of each of the breast carcinoma cell lines.

Cell Line Quality Control (QC)

Cell Morphology

- Control of the stages of cell line culture, harvest and preparation, through to controlled section thickness all contributes to the morphology seen in the final preparation.
- An acceptable preparation shows greater than 50% of cells with a rounded and centrally located nuclei, with minimal distortion of both nuclei and cell membrane. The inevitable dead cells within the preparation are excluded from interpretation.

Immunohistochemical Profile

- Fixation of the control cell lines in suspension enables a relatively homogeneous HER2 protein profile in all cell lines. Variation in HER2 expression is most commonly observed in the MDA-MB-175 (1+) and MDA-MB-453 (2+) cell lines respectively. When assessing control cell lines it is difficult to employ the percentage cut-off criteria that are designed to facilitate interpretation in tissue cases.
- Generally the relative homogeneity of a control cell line population, allows the majority of cells to demonstrate uniform HER2 staining at the appropriate expression level. However, in the case of the MDA-MB-175 and 453 (1+ and 2+) cell lines, IHC expression percentages may range from 30-90% of the viable cell line population, with the MDA-MB-175 (1+) cell line remaining incomplete with weak intensity and the MDA-MB-453 (2+) cell line remaining complete at weak to moderate intensity levels.

Core Density

- Regulated core density ensures viable cell numbers within the core population yielding consistent and reliable material for EQA assessment.
- Viable cells present should be in excess of 50% of the total field of view to allow visible verification of the staining profile.
- At Leica Biosystems, invasive breast carcinoma multi-tissue control slides demonstrating HER2 expression levels at 1+, 2+, 3+ are incorporated in to all HER2 cell line quality control runs to ensure that the control cell lines are a viable system and EQA tool in the diagnostic setting.
- Additional quality steps are performed by UKNEQAS ICC & ISH, with sections also stained using different commercially available HER2 IHC and ISH detection systems (e.g. Dako HercepTest, Oracle HER2 Bond IHC System, Ventana Pathway 4B5, Abbott Molecular PathVysion HER2 DNA Probe Kit: Figure 5). These stained sections allow UK NEQAS ICC & ISH to set the baseline by which the different commercial kits can be assessed.

Participant Gallery – UKNEQAS Control Cell Lines

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<th>HER2 Staining Profiles</th>
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<th>MDA-MB-175 (1+)</th>
<th>MDA-MB-453 (2+)</th>
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Specific Control Cell Line Characteristics

- Specific complete staining – moderate intensity, majority of membranes complete - overstained
- Non-Specific strong luminal surface staining (Not Interpreted) – overstained
- Non-Specific moderate luminal surface staining (Not Interpreted) – overstained
- Specific weak incomplete membrane staining (1+) – overstained

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Figure 6. MDA-MB-175 (1+) cell line demonstrating unique glandular-like luminal formation with correct HER2 IHC staining pattern highlighted. Stained with Dako HercepTest (K5204).

Figure 7. MDA-MB-175 (1+) cell line demonstrating unique glandular-like luminal formation with incorrect over stained HER2 IHC pattern highlighted. Stained with Dako Polyclonal (A0485) with pressure cooker antigen retrieval.

Cellular Morphology, IHC Profile

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Participant Gallery – In-house Tissue Controls

Acceptable Staining

- Acceptable Staining
- Unacceptable Staining

Figure 9. Examples of acceptable staining of HER2 IHC grades with Ventana Pathway 4B5 (A & B) and the Leica Oracle HER2 Bond IHC System (C & D) in participant in house breast cancer tissue controls

Figure 10. Examples of excessive cytoplasmic staining (A), tumour retraction artifact (B & C) and overstained normal breast epithelium in participant in-house breast cancer tissue controls

Conclusions

- Cell line controls, in the breast HER2 setting have become an integral part of both commercial assay and EQA monitoring. The consistency in both cell line protein and gene expression over extended time periods ensures that long term trends can be monitored; this is something that cannot be achieved through assessment of tissue alone. However, coupled with the use of in-house tissue control material this partnership ensures that EQA monitoring is reflective of both the individual, the laboratory and the overall testing population.

ICHC and FISH images provided courtesy of UKNEQAS ICC & ISH