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

**Part 1 – Cell Line Characterisation**

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**Introduction**

- Cell line controls in immunohistochemistry (IHC) and in situ hybridisation (ISH) have become established as a tool for monitoring assay performance. Their use has become increasingly prominent due to their application as system controls in prognostic and predictive assays, including the evaluation of HER2 status in breast cancer patients for whom Herceptin® is being considered.

- Whilst we acknowledge the application of control cell lines as analyte and external quality assurance (EQA) monitoring tools, it is important to emphasise that appropriate tissue controls, fixed and processed in the same manner as diagnostic materials, remain the gold standard in laboratory assay control. The use of control cell lines provides complementary and consistent monitoring for both commercial systems and EQA.

- The aims of this characterisation update are:
  - Outline the characterisation 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.
  - Describe how control cell lines can accurately reflect appropriate tolerances of laboratory based HER2 assays, ensuring they are a viable and constructive control device for UKNEQAS ICC & ISH participants.

**Cell Line Characterisation**

- Receptor load assessment of control cell lines is an important step in characterising cell lines as analyte controls. This verification step complements the subsequent protein and gene profile analysis, using the established diagnostic methodologies; IHC and FISH. Here we briefly describe the characterisation and analysis of 4 breast cancer cell lines using a flow cytometry based methodology.

**Figure 1a, b and c.** Cell line receptor load analysis using mouse monoclonal anti-HER2 antibody (clone CB11).

- 1a. Murine IgG coated calibration beads with 5 different antibody binding sites concentrations.
- 1b. 5-point standard curve created using calibration beads of known IgG antibody binding site concentration tagged with FITC-conjugated goat anti-mouse immunoglobulins.
- 1c. Separate suspensions of whole cells from each breast cancer cell line were fixed, then permeabilised to allow CB11 monoclonal antibody (mouse anti-human) to bind to the internal domain of the HER2 receptor. Following incubation and washing, cells were labelled with the secondary layer, FITC-conjugated goat anti-mouse IgG. The fluorescent signal was then quantified using flow cytometry and the signal plotted on the standard or calibration curve, to enable an approximation of receptor load per cell.

**Figure 2.** Illustrating monolayer cell growth, fixation in suspension, through to capture in an agarose matrix.

- Following appropriate harvesting, cells are fixed in 10% (4% v/v) neutral buffered formalin (NBF). NBF is also the recommended fixative for breast cancer cases through to paraffin wax.

**Table 1.** HER2 protein profile by IHC, gene status by FISH and corresponding receptor load by flow cytometry, taken from The Oracle HER2 Bond IHC System - Instructions for Use, Leica Biosystems.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>HER2 IHC Profile</th>
<th>HER2 Receptor loading</th>
<th>HER2 Copy Number</th>
<th>HER2:Chr17</th>
<th>HER2:Chr 17 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK-BR-3</td>
<td>3+</td>
<td>4.3x10^5</td>
<td>8.9x10^3</td>
<td>3.55</td>
<td>3.55</td>
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<tr>
<td>MDA-MB-453</td>
<td>2+</td>
<td>1.4x10^5</td>
<td>2.5x10^3</td>
<td>2.05</td>
<td>2.05</td>
</tr>
<tr>
<td>MDA-MB-175</td>
<td>1+</td>
<td>6.3x10^5</td>
<td>3.3x10^3</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>0</td>
<td>9.3x10^5</td>
<td>3.1x10^3</td>
<td>1.13</td>
<td>1.13</td>
</tr>
</tbody>
</table>

**Growth of Cell Lines**

- Many commercially available cell lines are adherent in culture and grow as a monolayer. Key features of this growth process include:
  - Providing nutrients and an environment tailored to the requirements of each cell line.
  - Limiting repeated passage of cells and using cells within a passage number window as close as possible to the passage number of the initial culture.
  - Harvesting cells mechanically as opposed to chemically.
  - Optimisation of the above processes (see Figure 2) enables the generation of control material which accurately reflects cell morphology and protein expression.
  - Long-term observation also enables monitoring of:
    - Recognition of a sudden genetic mutation that may result in altered HER2 gene/protein expression.
    - Increases confidence in the ability to supply control material of required specification.
    - Possible expansion of defined selection criteria windows through generation of increasing amounts of experimental data.

**Section Thickness Measurement (Interferometry)**

- Based on work published by Barker et al 2009, each UKNEQAS IHC and ISH HER2 cell line control slide is non-destructively tested using a Leica Biosystems patented white light interferometry system.

**Figure 3.** Uniform cell distribution and layout of the UKNEQAS control cell lines.

**Figure 4.** Topographical image of the UKNEQAS IHC and ISH HER2 cell line control on a glass slide as generated by white light interferometry. This technique provides an accurate method for consistent HER2 cell line control slide manufacture that stain reproducibly.

**This unique process ensures accurate section thickness is maintained, enabling consistency in control slide staining. This level of control is critical to achieving accurate HER2 assay validation and continuous batch performance.**

**References**