Cell Lines Are Non-Inferior to Tonsil as Controls for Ki-67 Assays When Measured by Image Analysis

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Background

Ki-67 is widely used for diagnostic and prognostic purposes. However, standardized evaluation is lacking. Reliable laboratory-developed tests require calibration controls and precise methods of interpretation.

To this end, we applied artificial intelligence-based image analysis to compare cell lines to tonsil tissue when serving as Ki-67 control material. We postulated that cell lines show greater consistency than tonsils and that cell lines are non-inferior for capturing changes in Ki-67 reactivity caused by protocol changes. We correlated these control materials to breast carcinomas in a variety of assay conditions typically altered in assay optimization, including antibody clone, dilution, incubation time, and pH of retrieval solution.

Materials & Methods

We made a microarray block containing 3 tonsils, 6 cell lines, and 4 breast carcinomas with a spectrum of proliferative indices. Six replicate slides were stained with MIB-1 or 30-9 clones on Dako Autostainer Link 48 or Ventana Benchmark Ultra stainers, respectively. MIB-1 and 30-9 concentrations were varied from 1:100 to 1:1600, or 1:1 to 1:8 (predilute).

Antigen retrieval was performed with low or high pH, and Ab incubation time was 15 vs 30 minutes (Dako), or 16 vs 32 minutes (Ventana). Slides were scanned on a Hamamatsu NanoZoomer S360. Images were analyzed using AI-based algorithms on the Visiopharm software platform.

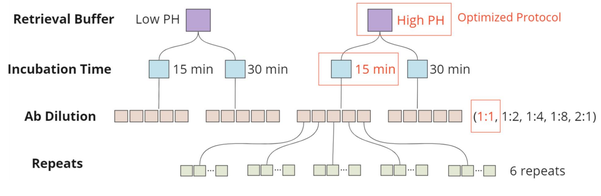
Materials & Methods

Mean proliferation indices and H-scores were generated for each sample. Breast carcinoma mean Ki-67 values were considered ground truth against which the controls were compared.

Statistical analysis was performed in R (2023.03.0+386). Each breast carcinoma was treated individually, as each represented a distinct clinically relevant expression range. To define the "change" in reactivity, we calculated the difference of the H-scores of each sample, in each assay condition, to the mean value from the validated assay. The tonsils and cell lines were correlated to each of the breast carcinomas.

To define the non-inferiority range, we calculated the mean 95% confidence interval (CI) of the tonsil samples' Pearson correlation rank coefficient (CIs calculated with Fisher's transform). To test each cell line for non-inferiority, we used the lower CI95% for the cell lines' correlation rank coefficient and compared them to the non-inferiority range individually.

Experimental Design

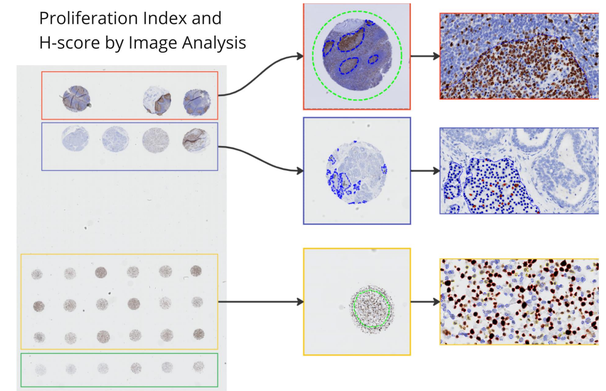


Results

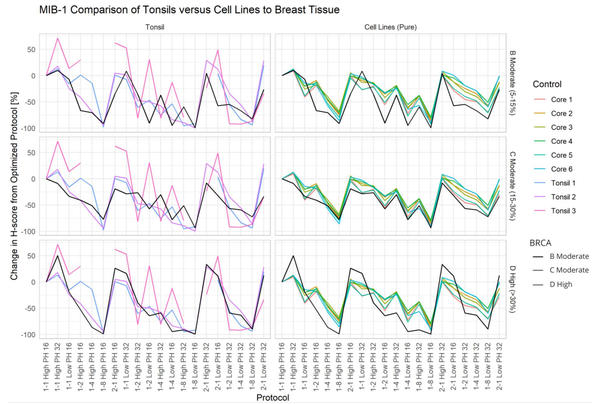
MIB-1: For breast carcinomas with moderate KI-67 indices (5-15% and 16-30%), all six cell lines were at least non-inferior to tonsils for capturing changes due to MIB-1 assay conditions. For the highly reactive breast carcinoma (>30 %), at least 3 cell lines (#3, #4, #6) showed non-inferiority.

30-9: For breast carcinomas with moderate Ki-67 indices of (5-15% and 16-30%), at least 3 cell lines (#1, #3, #4) showed non-inferiority to tonsils for capturing changes due to 30-9 assay conditions (data not shown). For the highly reactive breast carcinoma (> 30%), none of the cell lines show non-inferiority due to high CI95% correlation between tonsils and breast carcinoma (R=0.95).

Figures



Figures



Conclusions

1. Cell line cores show consistent Ki-67 reactivity as measured by IA using proliferation indices (PI) or H-scores.
2. Cell lines are non-inferior to tonsils for monitoring changes in Ki-67 assay sensitivity.
3. Ki-67 clones 30-9 and MIB-1 respond similarly to changes in assay conditions.