

Flow cytometric analysis of DNA content and cell cycle status in formalin-fixed, paraffin-embedded breast cancer specimens: improved assay using a core punch approach.

Genomic aberrations, including changes in DNA content, are a hallmark of human solid tumors. A simple method for gauging aneuploidy in formalin-fixed, paraffin-embedded (FFPE) tumor specimens could be of prognostic value and/or a useful marker for comparison with other tumor parameters. Currently, DNA content in archival specimens is assessed using 50 μ m sections cut from a FFPE tissue block. The sections are deparaffinized and treated with protease to disaggregate cells followed by staining of cell nuclei with a fluorescent dye and flow cytometric analysis. In this study, we have investigated an alternative method of sampling FFPE tissue blocks using a novel 1mm diameter core punch approach that samples tissues through the depth of the FFPE block. This method 1) allows specific targeting of tumor regions within a FFPE surgical block, 2) allows investigation of intra-tumoral heterogeneity, and 3) should result in more intact cell nuclei than are obtained from a microtome section. We are comparing alternative proteases (trypsin, protease XXIV), dyes (propidium iodide, DAPI, Sytox Green, SYBR Green) flow cytometers (Beckman Coulter®Epics XL-MCL, [Beckman Coulter, Brea CA]; BD™ LSR II [BD, Franklin Lake, NJ]) and alternative software analysis packages (ModFit LT™, Verity Software House; FCS Express, DeNovo, Inc.) to develop a standardized core punch method for flow cytometric assay for DNA content and cell cycle analysis. Twenty eight breast cancer samples are under investigation. The data show that the core-punch approach readily shows up aneuploidic cell populations and altered cell cycle profiles; tumors showing evidence of genetic instability and intratumoral heterogeneity have been detected.