RETHINKING CLONALITY USING MODELING APPROACHES

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A combination of experimental procedures, imaging, and probability estimation are typically used as evidence of clonality for the manufacture of a biotherapeutic product. In situations where the totality of evidence is unavailable, establishing a high statistical probability for monoclonality can help strengthen the argument for clonality. In this study, the probability of clonality was re-examined for the limiting dilution method using a combination of experimental and modeling approaches. A limiting dilution experiment was performed using a 50:50 mixed population of GFP-and RFP-expressing cells and the plates were imaged over a span of two weeks. The imaged cells were scored for clonality and double checked with fluorescence imager. Among all wells that had single colony-like growth on day 14 and a single cell-like image on day 0, a fraction of the wells were confirmed to have two colors on day 14 by fluorescence imaging, indicating the singe cell-like day 0 images for these wells were false reads. Considering the possibility of having 2 or more cells with the same color in a particular well, we estimated the worst case total possible number of wells with 2 or more cells on day 0. Moreover, assuming a Poisson distribution for limiting dilution, the recovery rate of any single cell that grew into a visible colony by day 14 was estimated. Our modeling analysis indicated that only a fraction of the wells with >2 cells on day 0 could grow into non-monoclonal colonies. If cells from any of the wells with single colony-like growth on day 14 and single cell-like image on day 0 were chosen as the final clone, the probability of monoclonality was estimated to be > 95% with a 95% upper confidence limit.