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Application Note

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Cell Culture Quality Control Assay

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Introduction

A large number of variables exist that alter the growth and function of cells in culture. Many sources of variability are largely uncontrollable because they are inherent to the stochastic processes in biological systems.[1] Other factors can be identified and controlled. Some key controllable factors are (a) poor CO_2 incubator performance due to lack of calibration and stability of temperature, humidity and CO_2 over time; (b) non-quantitative and/or inconsistent procedures for feeding and splitting cell cultures prior to running cell-based assays. The issues are inconsistent limits of cell density and feeding schedules, inconsistent cell density at the time of assay and changes in cell morphology; (c) alterations in media constituents due to lot-to-lot differences and alteration of component concentrations over time due to degradation; (d) differences in cell culture growth surfaces such as supplier variability, vessel surface treatments and lot-to-lot variability, and; (e) biological issues with cell lines caused by carrying cells in continuous culture for extended period of time which in turns increases the risk of phenotypic drift, contamination of infectious agents and cross contamination with other cell types. All of these controllable variables can adversely affect the results and interpretation of data obtained from downstream assays leading to lost time and a waste of expensive reagents. The National Chemical Genomics Assay Guidance Manual version 5.0 provides a good overview of what is required to set up high quality assays.[2]

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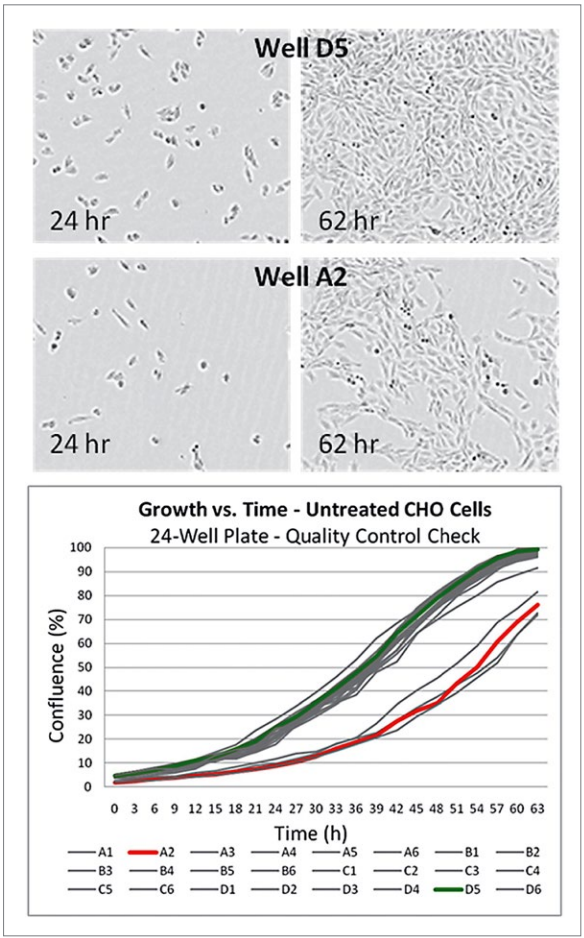
Approach and results

The IncuCyte® Live-Cell Analysis System is an automated microscope that provides a label-free, non-invasive method of monitoring cells at regular time intervals in their native environment; inside the cell culture incubator. Resulting data consists of objective, image-based growth metrics (Figures 1 and 2). The IncuCyte also has the ability to monitor hundreds of different types of cell culture vessels and the capacity to monitor several vessels at once. As cells become confluent, gene expression, protein expression and growth characteristics change (1, 2).

Pipetting errors during plate seeding result in different growth rates, different cell numbers and heterogeneous cell populations based on differential expression. The final effect of this is increased variability of cell-based assay data. Measuring confluence before utilizing cells for any assay is essential to ensure that experiments are run at consistent and optimized cell densities.



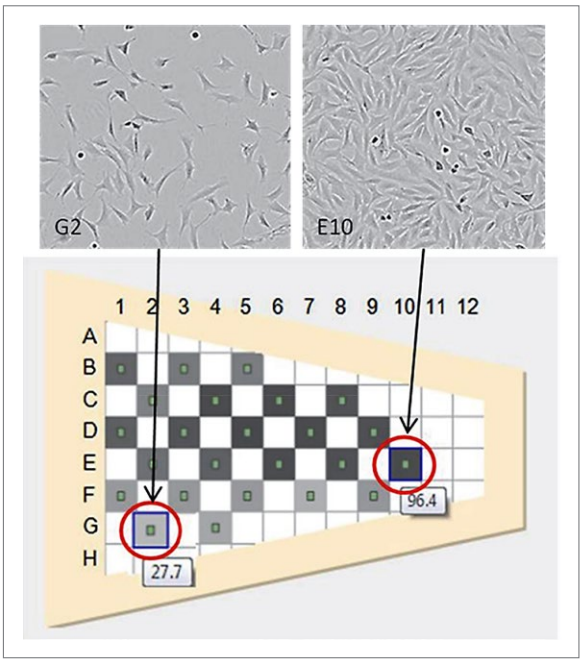
Figures 1 and 2. Images and data derived from the IncuCyte illustrating the effect of pipetting errors on growth: Wells in this plate were seeded at the same cell density and then checked prior to an endpoint assay for growth. Initial confluence for four wells was found to be ~50 % that of the median initial confluence for the plate; a variable likely induced by pipetting errors. Images from the two wells compared were retrieved from the database for visual inspection and correlation with the confluence data.

The breadth of data captured by the IncuCyte allows for a more accurate assessment of the overall cell monolayer, as well as spatial variations across a variety of vessels (Figures 3 and 4).

These variations can often be eliminated by changing technique, resulting in higher cell yields, less assay variation and homogeneous cell behavior.

A key element in controlling adverse variables is to standardize on objective metrics, thereby eliminating human subjectivity and interpretation. In sharp contrast to manual monitoring of the cell culture process, quality control monitoring with the IncuCyte automates the data capture and cell assessment process (Figure 5).

The IncuCyte provides the means to follow cells around-the-clock, and at precise, regularly scheduled sampling intervals. Images and data obtained are stored in the controller database and are accessible from any computer on the local network. In addition to supporting decisions required for the current culture process, this information can be retrieved months or years later to compare cell characteristics.



Figures 3 and 4. T-25 flask exhibiting uneven growth, often related to poor culture technique: Shown are two representative images from a single flask exhibiting confluence differences.

Conclusion

At Essen Bioscience, a Sartorius Company, we have pioneered the development of cell-based assays and assay technologies for years. With this experience we have come to appreciate what all researchers face when trying to control variables that can adversely affect cell-based assays. This experience led us to the development of the IncuCyte as a tool that can be used to improve the quality, predictability and reproducibility of cell-based assays. With the IncuCyte, you can follow and quantify cell growth over time, unveiling time dependent and transient phenomena. The IncuCyte is an essential cell culture quality control tool; providing a quantitative, objective, non-invasive, kinetic method of analysis on living cells in their native environment. Using the IncuCyte to document and monitor routine cell culture can improve cell-based assay quality and consistency.

References

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- MG Lampugnani, M Corada, P Andriopoulou, S Esser, W Risau and E Dejana (1997) **Cell confluence regulates tyrosine phosphorylation of adherens junction components in endothelial cells.** *Journal of Cell Science*, Vol 110, Issue 17 2065-2077.

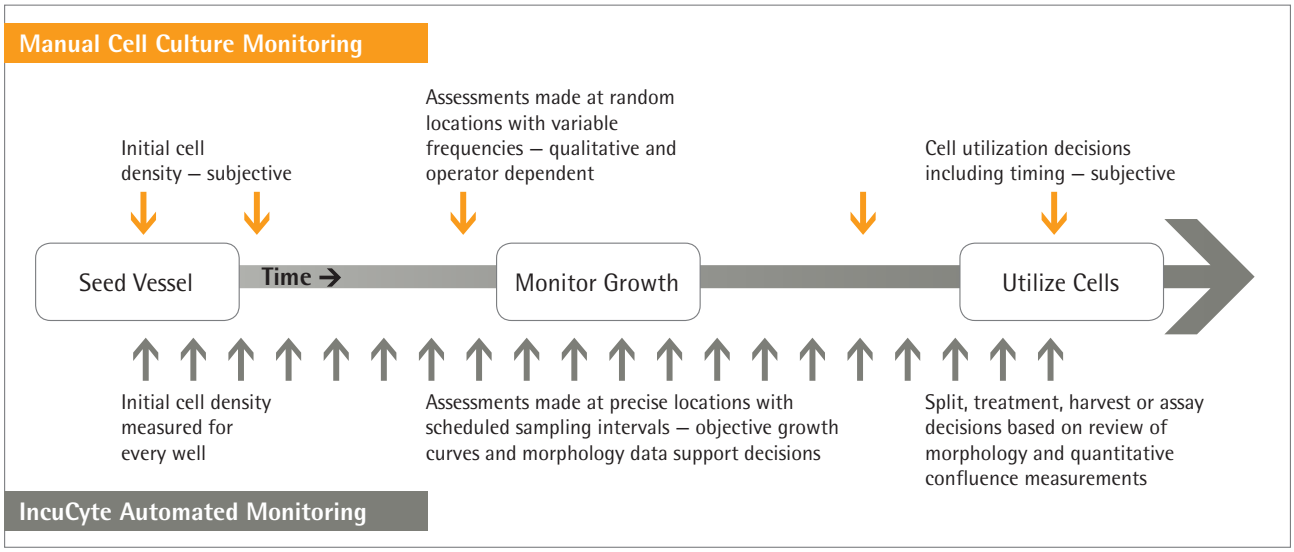

Figure 5. Comparison of cell culture monitoring methods: Vertical arrows represent interaction points with the cell culture for either method. Subjective decisions made during manual cell culture monitoring result in variability for cell-based assays.

About the IncuCyte® Live-Cell Analysis System

The IncuCyte® Live-Cell Analysis System automatically captures and analyzes images around the clock, providing an information-rich analysis that is easy to achieve. The IncuCyte resides inside your standard tissue culture incubator and accommodates a range of vessel ware, including flasks, dishes and microplates that can be imaged and analyzed in parallel. To request more information about the IncuCyte, please visit us at www.essenbioscience.com.

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