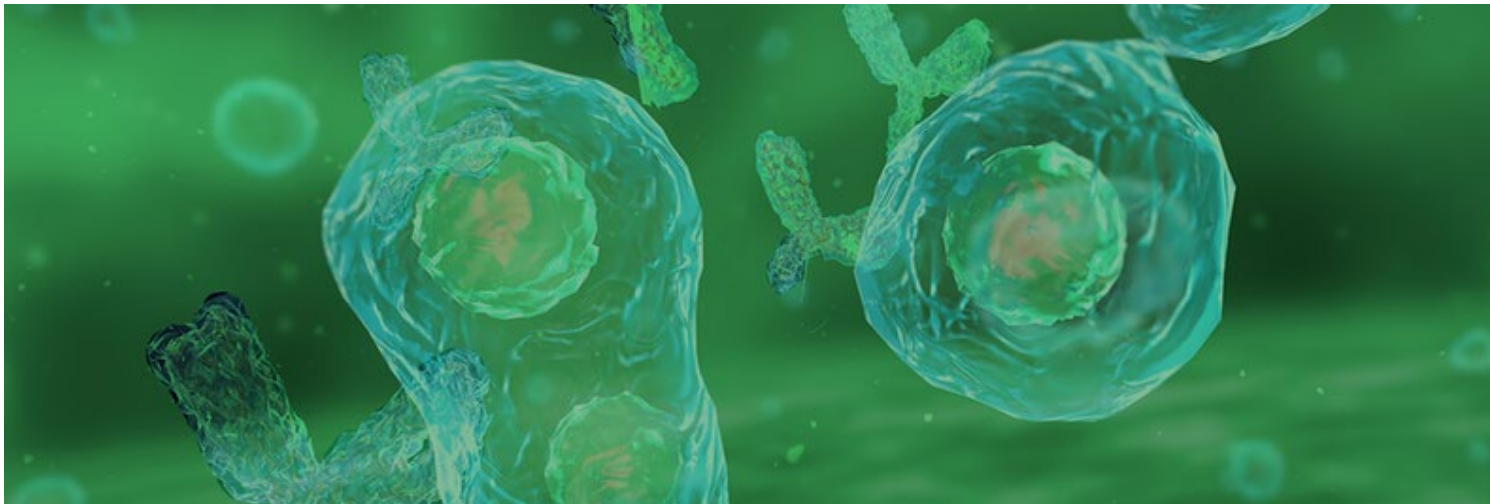


Analytical Power Tools Open Upstream Bioprocessing Bottlenecks

White Paper



The blockbuster success of antibody-based therapeutics for autoimmune diseases, inflammatory diseases and immunoncology accelerated the high stakes monoclonal antibody (mAb) development race. The market for therapeutic uses of mAbs was valued at USD 85.4 billion in 2015 and is expected to reach a value of USD 138.6 billion by 2024. In addition, mAb research is underway for therapies to treat neurodegenerative diseases, such as Alzheimer's and Parkinson's, as well as antibody/drug conjugates (ADC)—mAbs weaponized with radio-isotopes or cytotoxic agents for specific delivery to a cancer cell target.^{1,2,3}

Clone Selection: The Bioprocessing Bottleneck of Antibody Manufacturing

Clone selection is a significant upstream bottle-neck slowing bench-to-bedside development progress for new mAb-based therapeutics. Companies on parallel development paths—sometimes with overlapping therapies for similar targets—compete

to engineer productive cell lines that match target physical, chemical, or biological properties. Moreover, these cell lines must also be robust enough for the biomanufacturing environment.⁴ So, what is impeding the progress of new mAb-based therapies? Legacy screening technologies.

Legacy methodologies, such as ELISA or Bio-Layer Interferometry (BLI), are capable of evaluating only the immunoglobulin G (IgG) titer, but not the actual cells and cell health. Cells that appear to be productive because of high levels of IgG secretion may not be the most healthy and vigorous cells to take forward into the next step of the cell line generation process. However, the biggest bottleneck with legacy technologies, and headache for researchers, is the absence of integrated data analysis tools. Legacy systems require researchers to port data back and forth between multiple platforms—sometimes for weeks—increasing the possibility of error. Final output may not even be true data visualization, but merely a variety of spread sheets. Vigorous clone candidates are rare so this painstaking and capital/resource-intensive development process means clone discovery and optimization takes an average of 6 to 12 months.⁵ Additionally, since legacy methods only consider IgG concentration and not multiple cell attributes, uncertainty remains about whether the optimal clones were found.

Breaching the Bottle Neck with a Disruptive Fast-Track Antibody Screening Platform

Cy-Clone™ PLUS, a validated assay kit accelerates human mAb production cell-line generation, significantly reducing the time to industrial-scale productivity. The IntelliCyt® Cy-Clone PLUS assay combined with the IntelliCyt® iQue® Screener PLUS with ForeCyt® Software are the analytical power tools that inform rapid go/no-go decisions with multiparameter, high-content data from a single-path workflow. The microfluidics acquisition capability of the iQue Screener PLUS allows analysis of samples as small as 10 µL, in 384-well format, with zero dead volume. That means using less reagents while conserving a portion of the valuable IgG sample for future study. However, the real value proposition of

these analytical power tools is one-pass simultaneous reporting of the following additional cell health and cell number data points (Figure 1):

- Secreted IgG Quantification
- Total Cell Number
- Total Viable Cell Number
- IgG per Viable Cell
- IgG per Cell
- IgG per Cell per Day

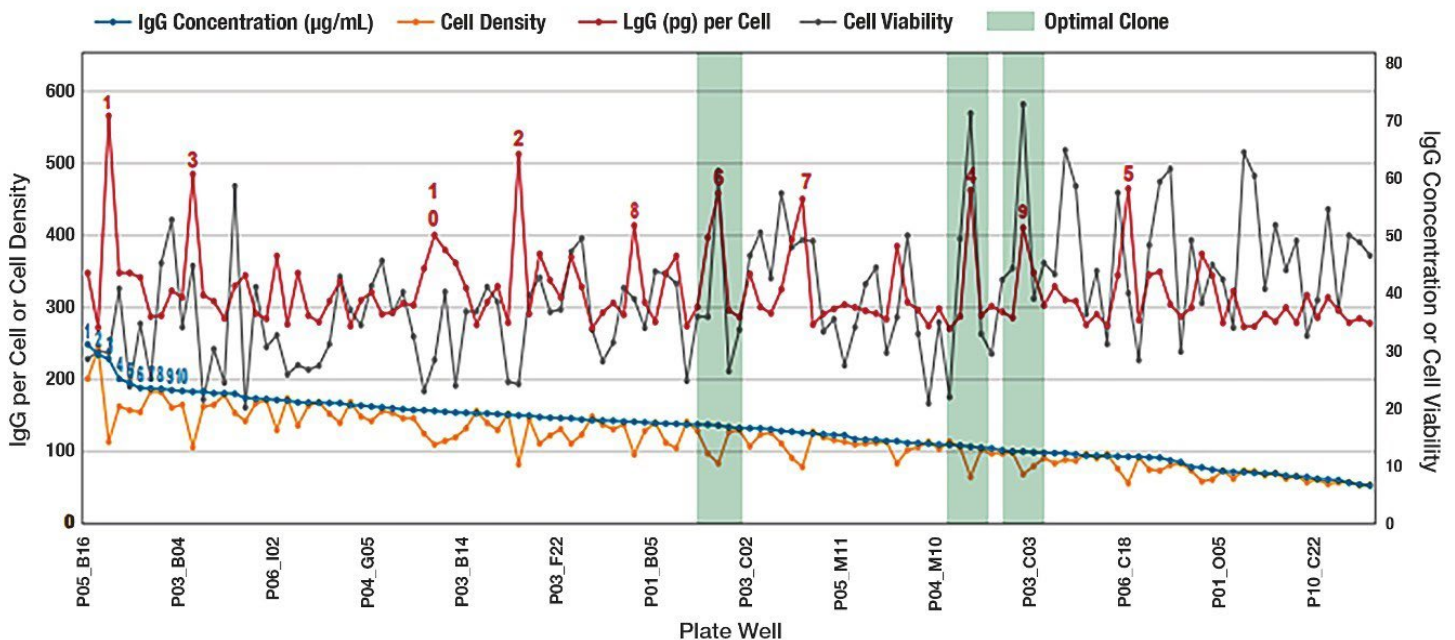


FIGURE 1. Example of simultaneously screening for IgG titer and cell health attributes.

The blue line represents IgG concentration and clone ranking that would be typical of legacy technologies measuring only IgG titer such as ELISA or BLI. A more complete story –and the advantages of the IntelliCyt integrated platform capability–is told in the additional data points relating to cell number and cell health. The red line is IgG quantitation per cell. The gray line demonstrates that while the IgG concentration declined there are still several very viable clones. The green shading shows clones that have high IgG titers on a per cell basis. These clones could be interesting candidates for downstream processing, and would likely have been excluded based solely on ranking by IgG titer. By combining separate time consuming steps in cell line screening processes, the IntelliCyt platform saves time, while providing valuable information on clone productivity.

Single-Path Workflow: Increased productivity by combining time intensive screening steps

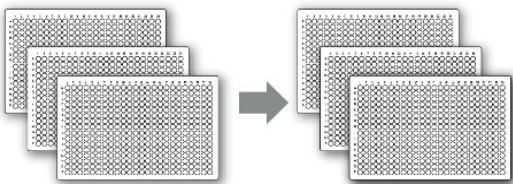
A protracted upstream process, coupled with limited time windows of patent exclusivity, makes time-to-production the difference between success and almost-made-it in bringing new therapies to market and improving patient outcomes. The tightly integrated workflow of the IntelliCyt Cy-Clone PLUS assay, iQue Screener PLUS, and ForeCyt Software streamlines workflow and provides real-time assay read-outs (Figure 2).

Cy-Clone PLUS addresses researchers' assay "wish-list": Direct transfer of cells and supernatant with no dilution steps achieves target concentration, saves time, and eliminates transfer errors that propagate across rows or columns.

As a no-wash assay, Cy-Clone PLUS eliminates tedious pipetting, centrifuging, and dispensing that results in cell and reagent loss. Wide dynamic range eliminates labor-intensive dilution of unknown samples. This streamlined design means a Cy-Clone assay set-up takes about an hour. Plus, there is no tedious preparation for data acquisition. A memory stick included in the kit has a pre-configured template complete with gates. Data acquisition begins immediately on the iQue Screener PLUS and takes 20 minutes for a 384-well plate.

Mix and Read Assay Workflow

Transfer **nondiluted** cell suspension



Mix in assay components



Read on the iQue Screener PLUS



FIGURE 2. Rapid, Streamlined Cy-Clone PLUS Workflow

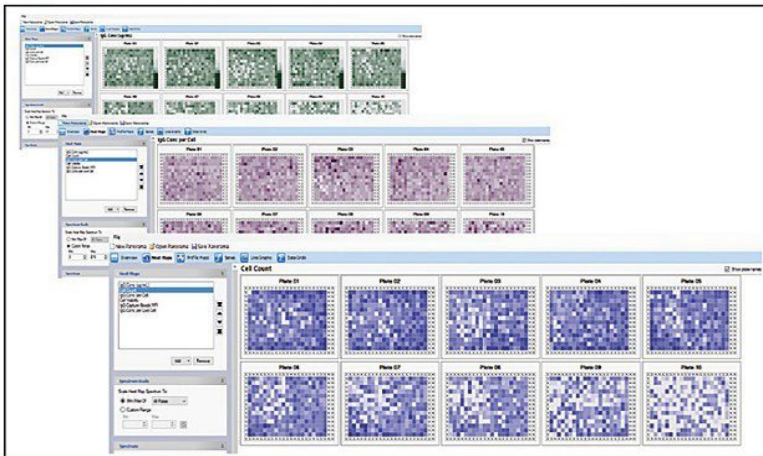
ForeCyt-Optimize, Analyze, Visualize, Realize...Faster!

ForeCyt Software produces actionable IgG clone data by generating cell-line plate heat maps, histograms, plots, dose response curves, and profile maps. A multi-plate analysis feature in the ForeCyt Software, called Panorama, generates an analytical "big picture" that automatically compares, identifies, and ranks the IgG clones at the screening campaign level (Figure 3) across multiple plates of an experiment, as well as multiple plates in an

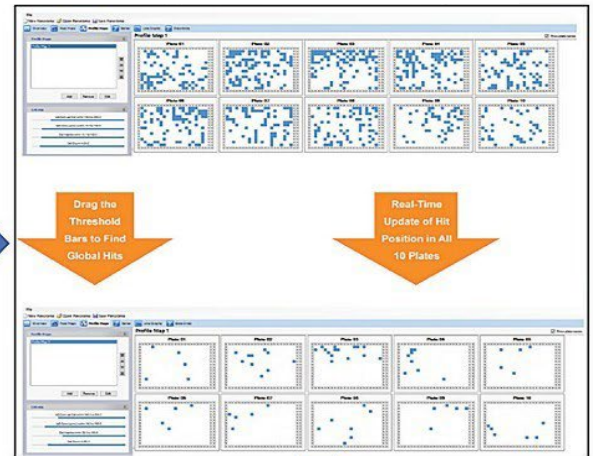
experiment over several days. In addition, criteria threshold slider bars adjust data on the fly for real-time "what if" analyses of plate data with the click of a mouse.

Panorama provides a capability to instantly dial-in an optimized set of IgG clones to move forward, eliminating the weeks or months of loading, reloading, and recalculating data required by legacy platforms.

Compare plates in heat maps



Identify hits in profile maps



Rank hits with correlated COAs

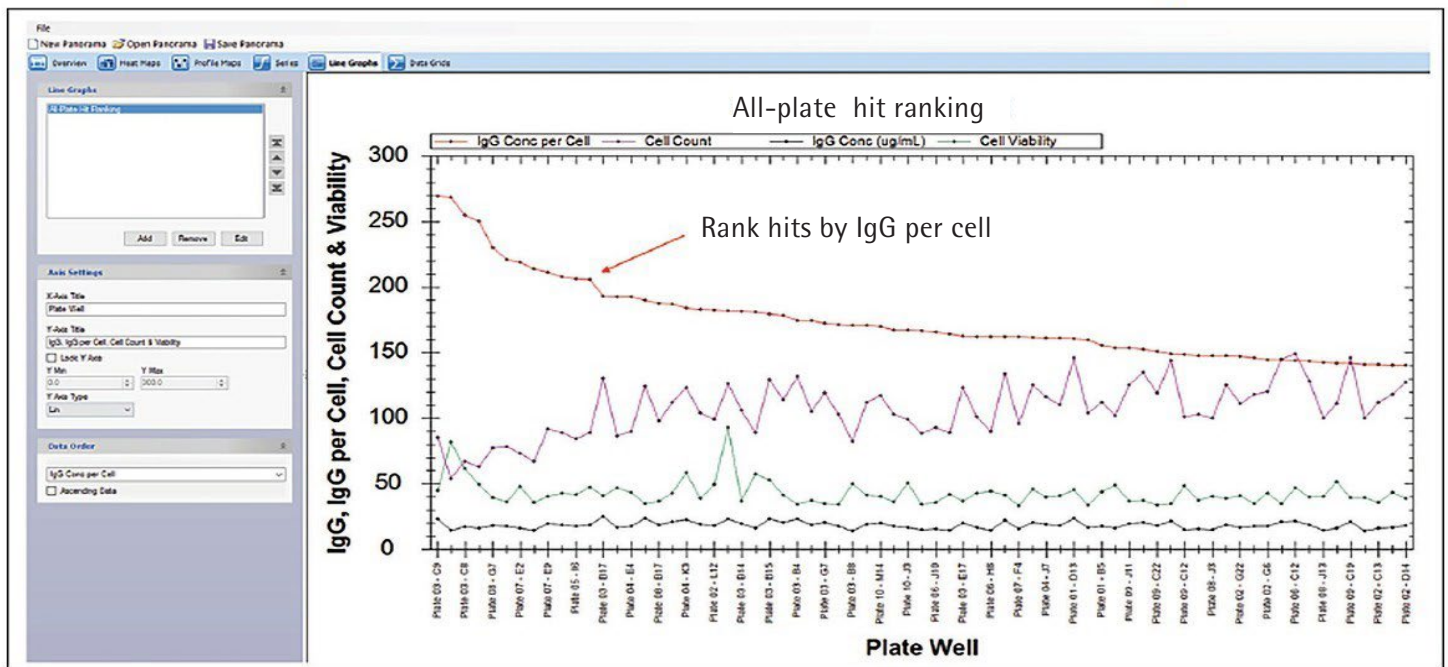


FIGURE 3. Cross-Plate Data Analysis Identifying Best Productive IgG Clones in a Screening Campaign

Multiple Upstream Bioprocessing Advantages

With antibodies holding so much therapeutic potential, the streamlined Cy-Clone PLUS, coupled with the iQue Screener PLUS and ForeCyt Software provides clear advantages for researchers over legacy technologies when characterizing important biomolecules. Besides being well-suited to manual processing, the iQue Screener PLUS is also designed to integrate with automated fluidics and robotic plate handling. Cy-Clone PLUS can also be incorporated into these bioprocessing activities: Cell health and IgG monitoring capabilities; media, feed, and bioreactor optimization; and process monitoring (Figure 4). As part of our

validated multiplex reagent portfolio, the PLUS in Cy-Clone PLUS means future multiplex capabilities to measure/monitor additional COAs including apoptosis, autophagy, proliferation, and antibody binding (Figure 4).

With mAb-based product development addressing diseases of such massive financial and societal implications, researchers using our analytical power tools will reach their goals faster and shorten the bench-to-bedside development path benefitting both patients and the bottom line.

Upstream processing Use the iQue® platform to measure IgG titers and other measurements of cell function

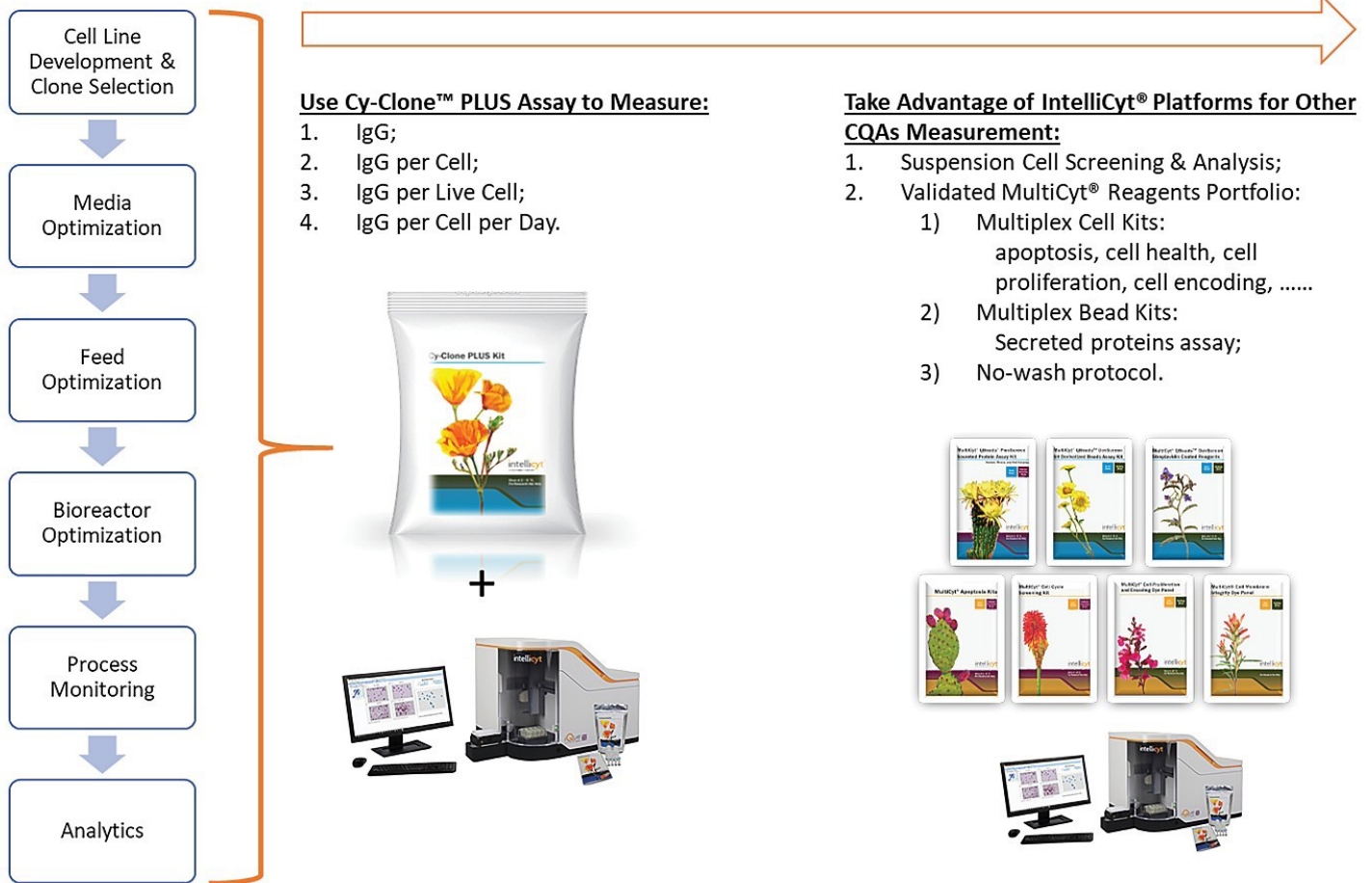


FIGURE 4. Use Cy-Clone PLUS and iQue Screener PLUS Platform to Measure Multiple Readouts in Whole Upstream Processing of Human IgG Therapeutic Proteins.

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