SARTORIUS

White Paper

2021

Keywords or phrases:
Monoclonal Antibodies (mAbs), mAb-based Therapeutics, mAb Development, iQue®, Advanced Flow Cytometry, Upstream Bioprocessing, Cell Line Development, Clone Selection, Antibody Manufacturing, iQue® Human IgG Tiiter & Viability Kit, Antibody Screening, IgG Clone, iQue Forecyt®

Analytical Power Tools Open Upstream Bioprocessing Bottlenecks

The blockbuster success of antibody-based therapeutics for autoimmune diseases, inflammatory diseases, and immuno-oncology accelerated the high stakes monoclonal antibody (mAb) development race. The market for therapeutic uses of mAbs 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 bottleneck 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 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 development 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 spreadsheets.

High-performance clone candidates are rare so this painstaking and capital- | resource-intensive development process means clone discovery and optimization take 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.

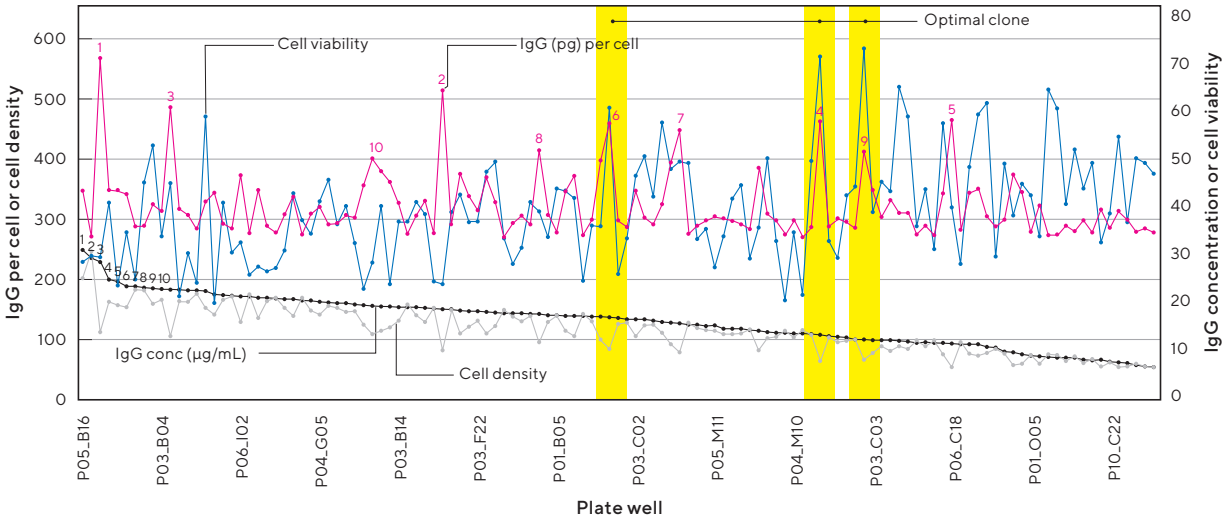
Breaching the Bottle Neck with a Disruptive Fast-Track Antibody Screening Platform

The iQue® Human IgG Titer & Viability Kit, a validated assay kit, accelerates human mAb production cell-line generation, significantly reducing the time to industrial-scale productivity. The iQue® Human IgG Titer & Viability Kit assay combined with the iQue® Advanced Flow Cytometry Platform with iQue 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® Advanced Flow Cytometry Platform 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

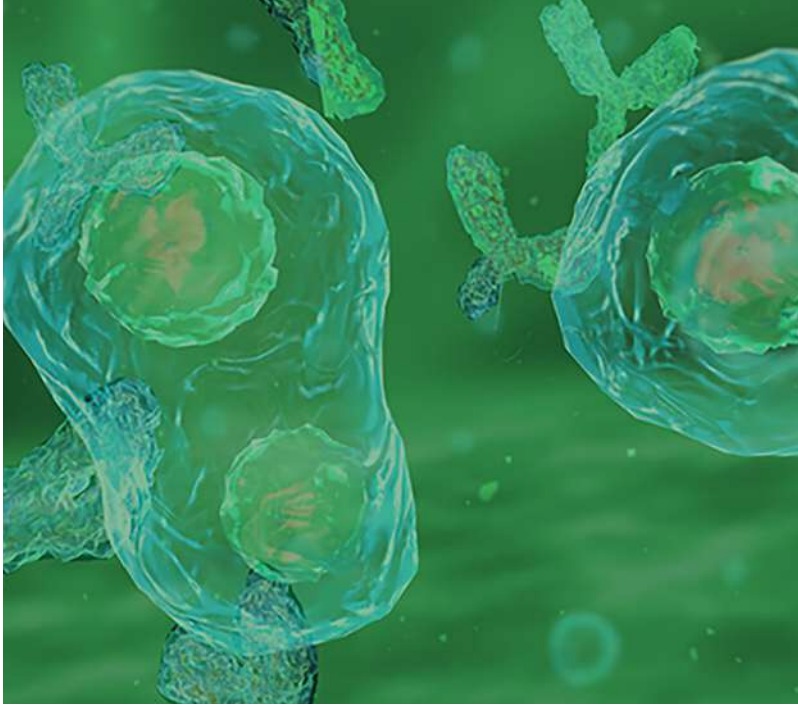
Figure 1

Example of Simultaneously Screening for IgG Titer and Cell Health Attributes



Note. The black line represents IgG concentration and clone ranking that would be typical of legacy technologies measuring only IgG titer such as ELISA. A more complete story—and the advantages of the iQue® integrated platform capability—is told in the additional data points relating to cell number and cell health. The pink line is IgG quantitation per cell. The blue line demonstrates that while the IgG concentration declined there are still several very viable clones. The yellow 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 iQue® platform saves time, while providing valuable information on clone productivity.

Single-Path Workflow: Increased Productivity by Combining Time Intensive Screening Steps

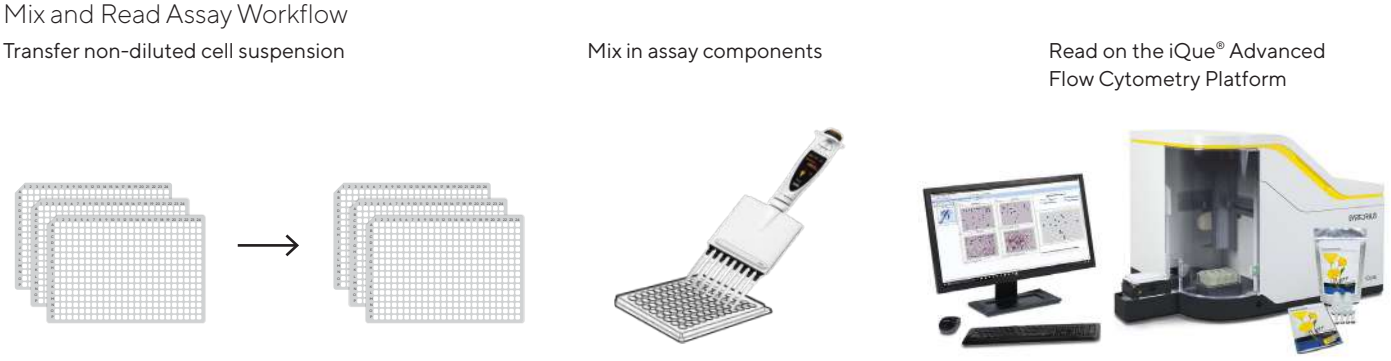


A protracted upstream process, coupled with limited time windows of patent exclusivity, makes time-to-production the difference between success and almost-made-it in bringing new therapies to market and improving patient outcomes. The tightly integrated workflow of the iQue® Human IgG Titer & Viability Kit assay, iQue® Advanced Flow Cytometry Platform, and iQue Forecyt® Software streamlines workflow and provides real-time assay read-outs (Figure 2).

The iQue® Human IgG Titer & Viability Kit addresses researchers' assay "wish-lists": 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, the iQue® Human IgG Titer & Viability Kit 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 an iQue® Human IgG Titer & Viability Kit 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® system and takes 20 minutes for a 384-well plate.

Figure 2
Rapid, Streamlined iQue® Human IgG Titer & Viability Kit Workflow



iQue Forecyt®—Optimize, Analyze, Visualize, Realize…Faster!

iQue 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 iQue 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 a click of the 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.

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 iQue® Human IgG Titer & Viability Kit, coupled with the iQue® Advanced Flow Cytometry Platform and iQue Forecyt® Software provides clear advantages for researchers over legacy technologies when characterizing important biomolecules. Besides being well-suited to manual processing, the iQue® platform is also designed to integrate automated fluidics and robotic plate handling. The iQue® Human IgG Titer & Viability Kit 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 iQue® Human IgG Titer & Viability Kit is compatible with future multiplex capabilities to measure | monitor additional CQAs 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.

Figure 4

Note. Use the iQue® Human IgG Titer & Viability Kit and iQue® Advanced Flow Cytometry Platform to measure multiple readouts in whole upstream processing of Human IgG therapeutic proteins.

References

1. Monoclonal Antibodies (mAbs) Market Size Worth $138.6 Billion by 2024. https://www.grandviewresearch.com/press-release/global-monoclonal-antibodies-market (accessed May 26, 2017)
2. Novel monoclonal antibodies show promise for Alzheimer's disease treatment. https://www.sciencedaily.com/releases/2015/07/150720154218.htm (accessed May 26, 2017)
3. Weiner GJ. Building better monoclonal antibody-based therapeutics. *Nat Rev Cancer.* 2015;15(6):361-370. doi:10.1038/nrc3930
4. Wiley StatsRef: Statistics Reference Online (2014)
5. Lai T, Yang Y, Ng SK. Advances in Mammalian cell line development technologies for recombinant protein production. *Pharmaceuticals* (Basel). 2013;6(5):579-603. Published 2013 Apr 26. doi:10.3390/ph6050579

North America
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
USA
Phone +1 734 769 1600

Europe
Sartorius UK Ltd.
Longmead Business Centre
Blenheim Road
Epsom
Surrey, KT19 9QQ
United Kingdom
Phone +44 1763 227400

Asia Pacific
Sartorius Japan K.K.
4th Floor, Daiwa Shinagawa North Bldg.
1-8-11, Kita-Shinagawa 1-chome
Shinagawa-Ku
Tokyo 140-0001
Japan
Phone +81 3 6478 5202