BioTechniques Spotlight

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eBook: Antibody discovery

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BioTechniques Spotlight

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Introduction

Antibodies are one of the most important classes of biologics in the life sciences. Their ability to bind specifically and tightly to target biomolecules makes them ideal therapeutics, and there are now over 160 antibody therapies approved for use to treat a wide variety of diseases, such as cancers, autoimmune disorders and infectious diseases.

Antibody discovery is a crucial first step in developing antibody therapeutics. Large numbers of antibodies are screened and assessed for their therapeutic potential; these programs rely on high-throughput technologies to quickly identify candidates. Discovery workflows begin with identifying thousands of antibodies against a specific target and then triaging them through a series of in vitro binding and functional assays to identify the top leads.

This eBook rounds up key features from our Spotlight on antibody discovery, including the various techniques and technologies utilized in antibody discovery, how high-throughput flow cytometry can be used for successful discovery campaigns and emerging role of artificial intelligence in antibody discovery.

Annie Coulson
Digital Editor,
BioTechniques
a.coulson@future-science-group.com

Interview

Developing immunodiagnostics to tackle antimicrobial resistance

Vaishali Verma (right) is an Assistant Professor in the Department of Biotechnology at Bennett University (Uttar Pradesh, India) who develops immunochemical reagents for the diagnosis of drug-resistant bacteria. In this interview, we talk to Vaishali about the role that antibody discovery plays in her research, the techniques she uses and how her work contributes to the fight against antimicrobial resistance.

Could you please introduce yourself and give us an overview of your research?

My primary research expertise lies in the field of antibody discovery and engineering using surface display technology, primarily phage display. With funding from the Science and Engineering Research Board (Department of Science and Technology, Government of India), I am currently working on producing recombinant mouse antibodies that would be used for developing immunodiagnostics to profile carbapenem resistance in drug-resistant bacteria. My goal is to contribute to the development of simple and effective diagnostic tools for the detection and management of drug-resistant bacteria, which pose a significant public health concern.

What techniques do you use for antibody discovery?

To discover antibodies, we employ the surface display technology called phage display. This technique involves the expression of a library of antibody fragments (scFv) derived from mice immunized with the target antigen on the surface of bacteriophages (viruses that infect bacteria). Subsequently, through a process called biopanning, the libraries are screened against specific target antigens to isolate high-affinity antibody fragments. The selected clones are further screened for specificity using ELISA, Western blot, etc. After isolating specific antibodies, we reformat them into full-length antibodies and express them in mammalian expression systems, for large-scale production of antibodies.

What challenges do you face in this research?

If the conditions for selection are not optimized, the resulting clones may lack specificity. To mitigate this issue, we design and optimize selection strategies based on the properties of the target antigen to reduce false positives during the selection process.

Additionally, a key challenge in antibody production is the associated costs. To address this, we are actively working on optimizing our expression vectors and conditions for expression to improve the efficiency and cost-effectiveness of the production process.

How could your research contribute to the fight against antimicrobial resistance?

The global emergence of carbapenem resistance in bacteria is a significant concern, and the slow pace of antibiotic discovery has resulted in limited treatment options. Therefore, our primary objective is to develop reliable, and user-friendly point-of-care diagnostic reagents to facilitate timely patient treatment and enhance clinical outcomes. By ensuring the controlled use of carbapenem drugs and effectively managing the spread of infection by quick screening, we believe that we can help address this critical issue and reduce its impact.

What's next for your research?

Our objective is to leverage our optimized antibody discovery technology to not just create effective diagnostic tools but also work towards the development of antibody-based therapeutic interventions to combat drug-resistant bacterial infections.

Is there anything else you'd like to add?

Amid the COVID-19 pandemic, scientists worldwide collaborated to rapidly develop vaccines to mitigate its impact. Similarly, antimicrobial resistance poses a significant concern, and it is my sincere hope that the global scientific community will continue to work collaboratively and take proactive steps to address this issue. As an antibody engineer, I am committed to contributing my best efforts towards combating this issue.

Interview

Antivenom intelligence? The role of AI in developing snakebite treatments

We spoke to Tim Jenkins (right), an Assistant Professor at the Technical University of Denmark (DTU; Copenhagen, Denmark), about his research incorporating artificial intelligence into antibody discovery programs to develop new snakebite antivenoms.

Annie Coulson, Digital Editor, *BioTechniques*

Snakebites present a neglected public health issue in many tropical and subtropical countries – an estimated 5.4 million people are bitten by snakes each year, and roughly half of those people are injected with venom. Between 81,000 and 138,000 people die as a result of snakebites, while around three times as many are left with permanent disabilities [1].

Although antivenoms exist, there are many barriers to making them safe, effective and accessible to those who need them. Current antivenoms are made following a 100-year-old method of injecting a venom of interest into a production animal, such as a horse, waiting up to a year for the animal's immune system to generate antibodies and then collecting the blood plasma from the animal and purifying it. "It works, but it has a lot of downsides," Tim explains. The resulting antivenom is not tailored, so it might not target the most clinically relevant toxins and can cause adverse reactions; it's not pure, so large quantities are required for successful treatment; and the manufacturing pipeline is lengthy, and upscaling is challenging, driving up the cost. These factors make snakebite a huge socio-economic burden, impacting those in poorer, rural communities most. "It can cost a farmer in Africa more than he makes in a year to pay for just the vials of antivenom, not even the hospital treatment."

Researching and developing new snakebite treatments that address these challenges is difficult, mainly because snakebites are almost absent from the global health agenda, meaning funding is limited. "If we are talking about treating all snakebite across the world, we have about 2000 toxins that we need to potentially neutralize. How do we figure out which ones are the most important? How can we develop more effective antibodies and make the product cost effective and affordable to those most in need?"

Same same, but different

To develop new snakebite treatments in the most cost-effective way possible, Tim and his colleague Andreas Hougaard Laustsen-Kiel, a Professor at DTU, decided to use next-generation treatments that have been developed for other more well-funded diseases, such as cancer and HIV, and apply these to snakebite.

Specifically, they are developing broadly neutralizing recombinant monoclonal antibodies utilizing *in vitro* antibody discovery technologies to identify specific antibodies against snake venom toxins [2]. "Phage display is our bread and butter. We established this together with one of the inventors of phage display, John McCafferty. Over the last year, we also started implementing yeast display, which is a different *in vitro* technology. We now combine these two technologies for their different benefits."

Phage display may be their current bread and butter, but computational tools really take the cake.

Computer says yes

During the COVID-19 pandemic when access to labs was restricted, Tim was pushed back into the next-generation sequencing roots of his PhD and started applying computational ideas to antibody discovery. "That's where my research group that I started about a year ago is positioned – at the nexus between lab-

Antivenom intelligence? The role of AI in developing snakebite treatments

based *in vitro* science and more computational in silico aspects of science." In the last 6 months, his group has leaned into generative antibody design, which was sparked by recent publications from David Baker's group at the University of Washington in Seattle (WA, USA). Baker and his excellent team have pioneered methods to predict and design protein structures and recently developed a powerful new tool that can be used to design proteins by combining structure-prediction networks and generative-diffusion models, called RoseTTAFold (RF) Diffusion [3]. The tool is incredibly effective – previous design methods required hundreds of thousands of variants to be tested before finding one that performed as desired; however, with RF Diffusion, 100 designs would be tested and 10% could be viable candidates. "That blew my mind. We reached out to the Baker Lab in early January and asked if they might be interested in collaborating. Fortunately, we were able to convince them that snakebite was a cool area to try this tool out in."

The tool worked, and within four weeks of using it, the collaboration with Susana Vazquez Torres, a grad student at the Baker Lab, had yielded binders against toxin groups that they had been struggling with in the lab for years. "AI is now starting to really revolutionize science. It's been on the horizon for 5–10 years now, but we haven't really seen anything transpire. But what we have seen over the last year and what we are going to see over the next 2 or 3 years is going to change the way that biotherapeutics will be discovered, developed and manufactured."

What happens next?

So, what happens when these entirely in silico proteins are taken to the clinic? Although the team know that the proteins are good binders, they need to establish how immunogenic they are and if these novel structures cause any unwanted side effects that they haven't been able to predict. Tim isn't too worried about this, as the protein suite that these tools have learned from are all real, published proteins and should be reasonably safe to work with.

Tim is more concerned about what the landscape will look like if they go to regulatory approval. "When you are developing something for cancer, you usually have the money, so you can spend time waiting and figuring these things out. But in the field of snakebite envenoming, you have to be so cost effective that you can't be wasting precious time and need clear regulatory guidelines for these new molecules as soon as possible."

Money, money, money

With factors out of their control, like government approval and distribution, driving up costs, what is the team doing to ensure these antivenoms are cost effective? The main factor is developing binders that are broadly neutralizing [4], meaning that one binder can work against a range of different toxins that are still similar enough in structure to be recognized. "If you manufacture 10s of antibodies, it's exponentially more expensive than manufacturing a handful in the same product. Driving down the number of different antibodies we need to produce in the same batch for one antivenom will drive down the cost of manufacturing, which will help make the product more affordable."

Looking to the future

For Tim, the next challenge is harmonizing all the techniques him and his team are currently using, both in the lab and computationally, with automation. "A lot of our efforts are focused on how we miniaturize assays, scale them up, make them high throughput and automate them so we can link these powerful computational tools to the lab as effectively as possible."

This will help achieve Tim's dream of having a semi- or fully automated setup for designing proteins on the computer: "This would mean that for a new viral outbreak, we take the sequence of the pathogen and within a day, we could design a library of different binders against these pathogens. Within that same day, we might even be able to synthesize the DNA,

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express these binders and possibly test them. So, go from a pathogen sequence to a binder that can go into more sophisticated, either functional validation or maybe even animal settings within a few weeks time using automation technologies. I think that's the direction the field has to move in and we're already beginning to see that."

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High Throughput, Multi-Parametric Analysis Accelerates Antibody Discovery Workflows

Kirsty McBain[1], Daryl Cole[1], Julie Lovchik[2], Ben Tyrell[3], Clare Szybut[1]

[1] Sartorius UK Ltd., Units 2 & 3 The Quadrant, Newark Close, Royston Hertfordshire SG8 5HL UK
[2] Sartorius Corporation, 5700 Pasadena Avenue NE, Albuquerque, NM 87113 USA
[3] BioOutsource, Glasgow, UK

Introduction

Therapeutic monoclonal antibodies (mAbs) are one of the fastest-growing classes of drugs, targeting many disease areas including cancer, autoimmune disorders, chronic inflammation, and infectious disease. In oncology, mAb-based therapies are used to target solid tumors and blood cancers; for example Trastuzumab is an anti-Her2 mAb used to treat Her2-positive breast cancers, and Rituximab is an anti-CD20 mAb, used as part of combination therapy to treat non-Hodgkin's lymphoma and chronic lymphocytic leukemia.

Antibody discovery programs rely on high throughput technologies to quickly identify candidates with therapeutic potential. The discovery workflow using hybridoma or display technologies begins with identifying thousands of antibodies against a specific target, and then triaging them through a series of *in vitro* binding and functional assays to identify the top leads. These leads are then scaled up and analyzed further before preparation for clinical use. Screening methods that can analyze multiple attributes quickly and effectively early on in development are key to a successful discovery campaign.

Evaluating antibodies for the desired therapeutic mechanism of action (MoA) is an important part of the antibody screening strategy. For instance, binding and rapid internalization are desirable properties for antibody-drug conjugates (ADCs), where cytotoxic agents are delivered into cancer cells. In contrast, antibody internalization is less important when the goal is to induce antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP), which depend on cell receptors binding to the Fc portions of antibodies to induce an effector response.

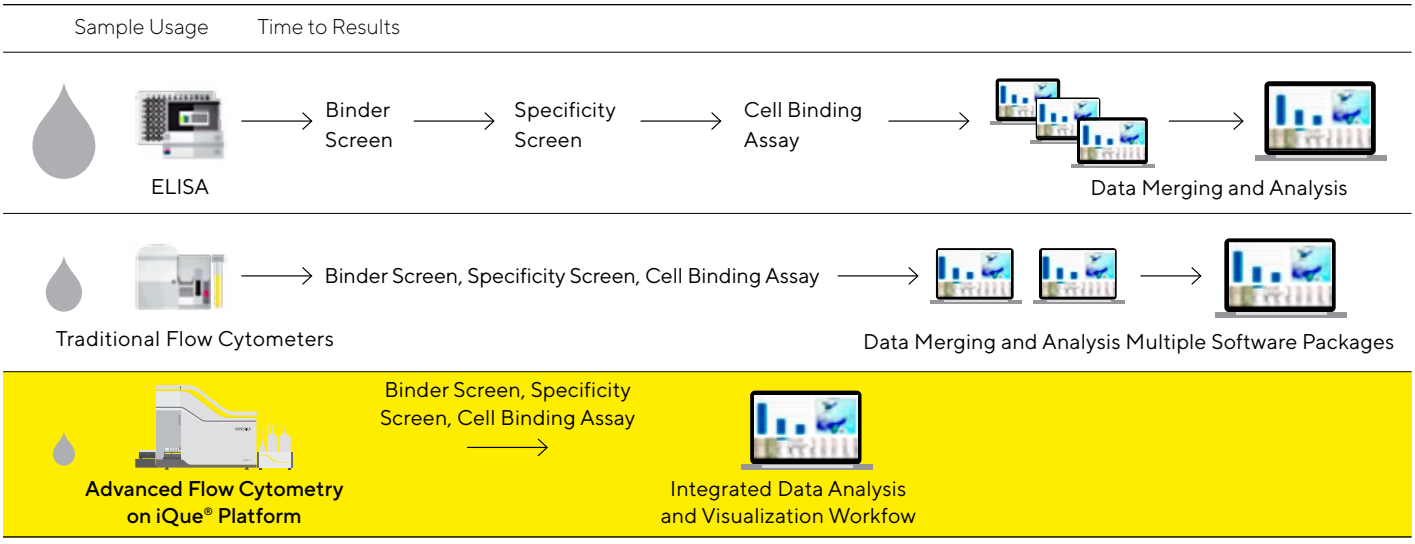
Find out more: https://www.sartorius.com/en/products/flow-cytometry

Traditionally, antibody screening workflows have relied on conventional flow cytometry, enzyme-linked immunosorbent assays (ELISA), and microscopy techniques for these characterizations (Figure 1). However, these methods have limitations: (1) they can be labor-intensive, (2) they may not support high-throughput applications, (3) they do not allow for direct antibody comparison, and (4) they need large amounts of reagents.

In this white paper, we present the unique benefits of iQue® advanced high-throughput flow cytometry in the antibody discovery workflow. Using predominantly studies with Her2-positive and CD20-positive cell models, and anti-Her2 and anti-CD20 mAbs, we show how this technology provides quantitative data on binding, antibody internalization, ADCP, ADCC and T cell activation.

Figure 1

Comparison of Sample Usage and Time to Results in Typical Biologics Workflows



Note. Comparison of sample usage and time to results in typical biologics workflows using ELISA, a conventional flow cytometer and the Sartorius iQue® Advanced Flow Cytometry Platform.

Antibody Binding

The first stage of an antibody discovery workflow is the initial screening of antibodies for binding and neutralization. Flow cytometry is often used to measure antibody binding and functionality. While flow cytometry has advantages over ELISA with respect to multiplexing capabilities, its low throughput is not ideal in the biopharmaceutical setting. Additional challenges with traditional flow cytometry assays are the large sample volumes, slow sampling times and tedious data analysis.

By contrast, the iQue® Advanced Flow Cytometry Platform is specifically designed for high-throughput, multi-attribute screening and provides many features that help streamline workflows and shorten time-to-results:

- Cells and beads can be simultaneously resolved in a single experiment, enabling quantitation of secreted proteins in parallel with cell-based analyses.
- Multiplexing capabilities refine the screening workflow, replacing multiple assays and platforms with one system.
- Experiments can be performed using small sample volumes, reducing associated reagent costs and preserving precious material.
- Continuous plate loading is enabled through connection with any automation system.

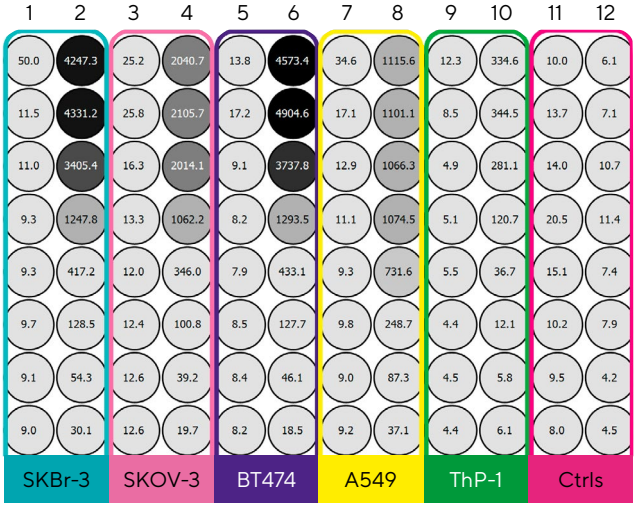
- Plate-based iQue Forecyt® analysis software and dynamic data visualization tools simplify analysis of large, complex data sets.
- Usability features, such as automated QC and instrument cleaning protocols, help save time.
- Optimized reagent kits featuring pre-templated analysis and simplified workflows streamline assays and data analysis, reducing time to actionable results.

In Figure 2, we used the iQue® Advanced Flow Cytometry Platform to measure the binding activity of several antibodies, including the anti-Her2 Herceptin antibody, with three Her2-positive (SKBr 3, SKOV 3 and BT 474) and two Her2-negative (A549 and ThP 1) cells. In this study, the serially diluted antibodies were incubated with cells prior to secondary labeling with a fluorophore-conjugated antibody. The binding dose-response curves were generated by using the mean fluorescence intensity (MFI) increase. Data visualization using the iQue Forecyt® software provides a heat-map representation of binding activity across the whole plate (Figure 2A-B). Histogram plots of single cells show binding activity for the Her2-positive cells, but not the secondary antibody-only or unstained controls (Figure 2C). Figure 2D shows dose-response curves for Herceptin binding to Her2-positive cells, while no binding activity was observed for the control samples.

Figure 2

High-Throughput Antibody Binding Analysis in Adherent Cell Types

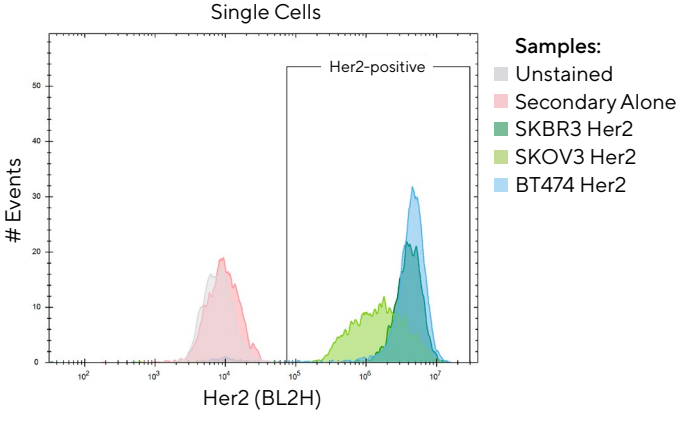
A.



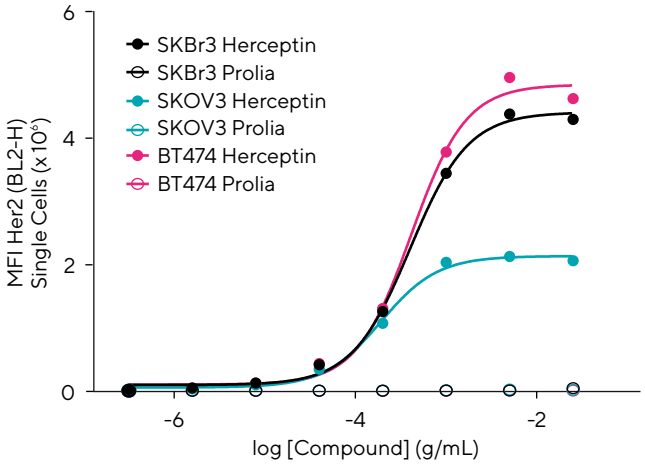
B.

Column	Antibody	Target
1, 3, 5, 7, 9	Prolia	RANKL
2, 4, 6	Herceptin	Her2
8	Erbitux	EGFR
10	Actemra	IL-6R
11	No 1° Ab	–
12	Unstained	–

C.



D.



Note. Various therapeutic antibodies, including Herceptin (anti-Her2) were used to demonstrate binding activity on the iQue® Platform. Five adherent cell lines were included on the plate, three Her2-positive (SKBr3, SKOV3 and BT474) and two Her2-negative (A549 and ThP1). Cell lines were seeded into a 96-well plate and serial dilutions of the antibodies were performed before being added to the plate, followed by the addition of the conjugated secondary antibody. The cells were carefully detached and resuspended prior to analysis. (A) Heat map illustrating MFI values in relation to antibody serial dilutions. (B) Table describing antibody location. (C) Histogram representative of Her2-positive cells when incubated with Herceptin. (D) Concentration response curves for Herceptin binding to Her2-positive cell lines.

Antibody Internalization and Viability

Antibodies that specifically bind to cell surface antigens induce endocytosis, which causes internalization of those antibodies. Antibody internalization (ABI) has many applications in drug development, such as delivery of cytotoxic payloads via ADCs.

Monitoring ABI and its associated kinetics is one of the key attributes when designing ADCs. There are numerous factors that can affect internalization kinetics, for example the epitope on the target antigen, affinity of the ADC-antigen interaction, and intracellular trafficking. Evaluating these factors is critical for optimizing mAbs during development.

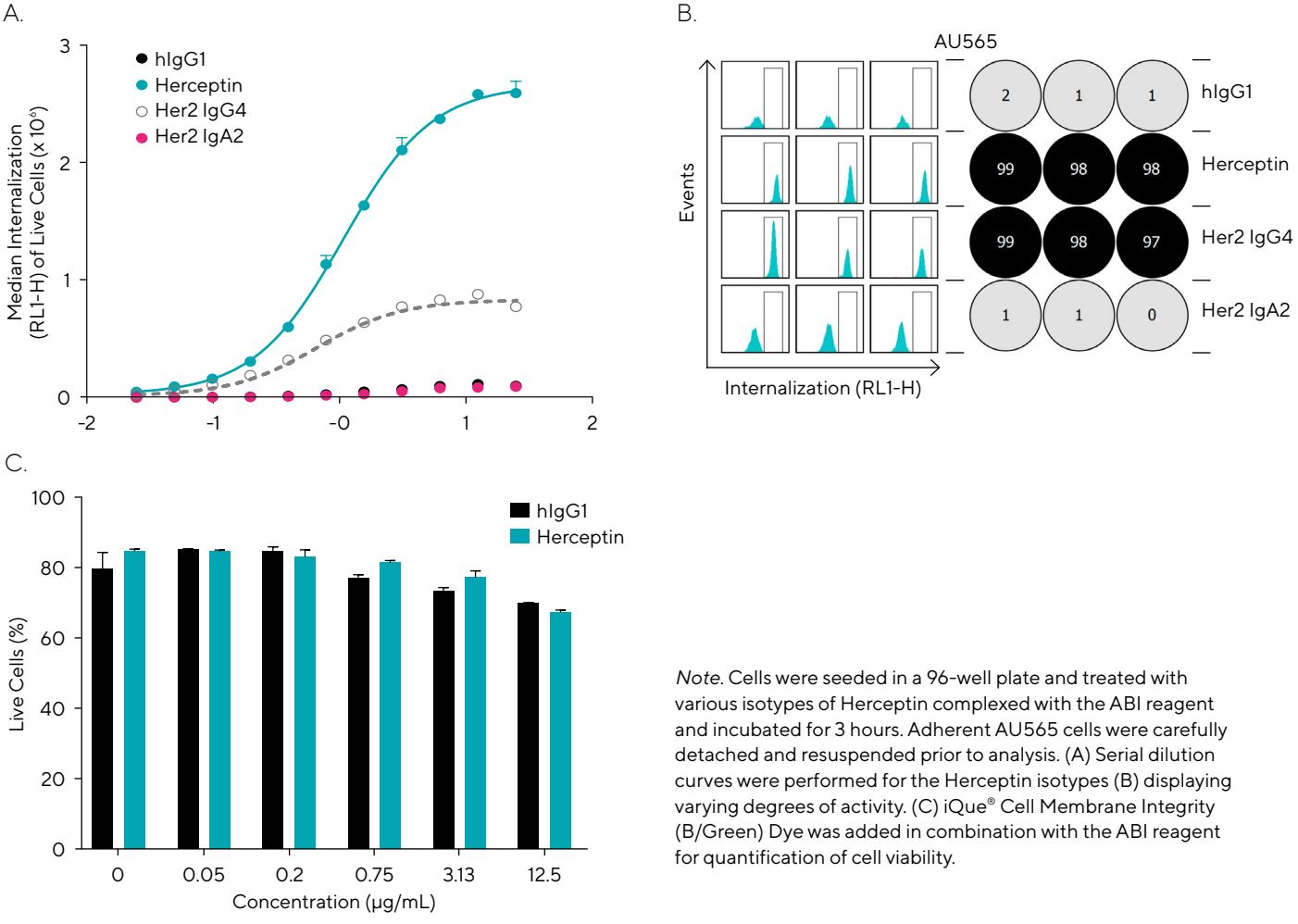
The iQue® Advanced Flow Cytometry Platform enables high-throughput measurement of ABI in suspension or adherent cells using a streamlined workflow coupled with easy data analysis and visualization on the iQue Forecyt® software. This assay uses a novel pH-sensitive fluorescent probe and a one-step, no-wash protocol to label isotype-matched antibodies. Once internalized and processed into the acidic endosome and lysosome pathway, the labeled antibody generates a fluorescent signal. ABI is quantified as the MFI of probe in live cells.

We used the iQue® Antibody Internalization Kit to measure internalization of Herceptin (anti-Her2) in adherent Her2-positive cells (AU565 cells) on the iQue® Platform (Figure 3). The iQue® Cell Membrane Integrity (B/Green) Dye and antibodies complexed with ABI reagent were added to cells that were seeded in a 96-well plate. Following incubation, adherent cells were carefully detached and resuspended prior to analysis. Our data shows concentration-dependent internalization of Herceptin, with concurrent verification of cellular viability.

We also evaluated internalization of Herceptin antibody isotypes (Her2, IgG4 and Her2 IgA2) using the same ABI assay. Here, we saw differential activity between isotypes with the Her2-positive cells. We did not observe internalization with Her2-negative cells (data not shown).

Figure 3

Internalization of Herceptin Antibody Isotypes in Adherent Her2-positive AU565 Cells



Note. Cells were seeded in a 96-well plate and treated with various isotypes of Herceptin complexed with the ABI reagent and incubated for 3 hours. Adherent AU565 cells were carefully detached and resuspended prior to analysis. (A) Serial dilution curves were performed for the Herceptin isotypes (B) displaying varying degrees of activity. (C) iQue® Cell Membrane Integrity (B/Green) Dye was added in combination with the ABI reagent for quantification of cell viability.

ADCP Measurement in Adherent Cells

ADCP and ADCC are two of the mechanisms used by therapeutic mAbs to clear cancer cells. In ADCP, mAbs engage Fc receptors on phagocytic immune cells via their constant region, while their variable region binds specifically to receptors that are over-expressed on tumor cells. Powerful, high-throughput techniques for characterizing ADCP are crucial to guide optimization of mAb constant and variable regions. However, traditional flow cytometry and microscopy methods for measuring ADCP involve lengthy protocols and complicated data analysis.

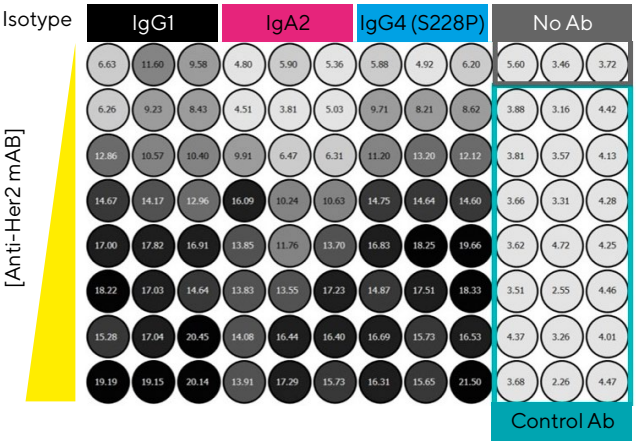
Here, we used the iQue® Human Antibody Dependent Cellular Phagocytosis Kit and the iQue® Platform to measure ADCP of adherent tumor cells (Figure 4).

Adherent Her2-positive AU565 breast cancer cells were incubated with anti-Her2 mAbs and PBMCs. ADCP was induced by three anti-Her2 mAb isotypes: anti-Her2-IgG1 (Trastuzumab), anti-Her2-IgA2 (a native isotype with reduced ADCP activity relative to the IgG1), and anti-Her2-IgG4 (S228P, an engineered isotype that also has reduced ADCP activity relative to IgG1). All three isotypes induced a concentration-dependent increase in ADCP activity relative to the control antibody. As expected, the highest maximal ADCP response (19 ± 4%) and most potent response (EC_{50} = 6.6 ng/mL) was measured with the IgG1 isotype (Figure 4C). These data show that this assay is suitable for quantifying mAb-induced ADCP of adherent targets.

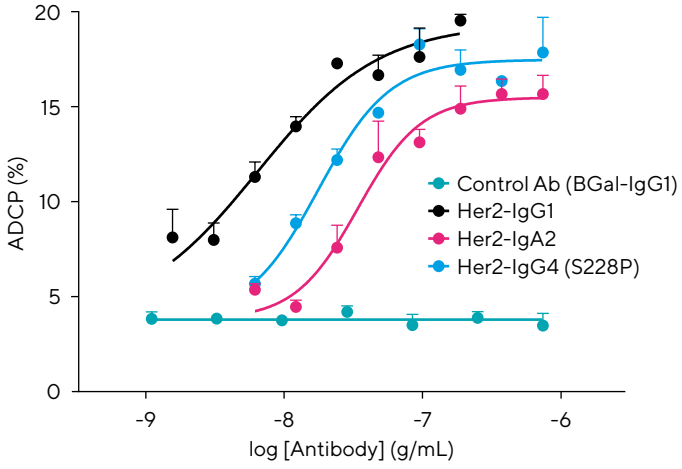
Figure 4

Pharmacological Differences in ADCP Induction Can Be Quantified With Anti-Her2 mAbs and Adherent Target Cells

A. ADCP (%)



B. ADCP Response to Anti-Her2 mAb Isotypes



C. Anti-Her2 mAb Isotypes with EC_{50} Values and Maximal ADCP

Isotype	ADCP[1]	EC_{50} (ng/mL)	Maximal ADCP (%)*
IgG1	+++	6.6	19 ± 4
IgA2	+	35.4	15 ± 2
IgG4 (S228P)	+	18.2	17 ± 1

** Best fit top of curve*

Note. Adherent AU565 cells (10K/well) were seeded in a 96-well plate with PBMCs (20:1 E:T) and ADCP was stimulated with various isotypes of anti-Her2 mAb (IgG1, IgA2 and IgG4 (S228P). ADCP was quantified using the iQue® Human Antibody Dependent Cellular Phagocytosis Kit and the iQue®. (A) Heat map with % ADCP with each Her2 mAB isotype. No antibody or a Bgal-IgG1 mAb were included as negative controls. (B) Concentration response curves for ADCP response to anti-Her2 mAbs. (C) Table describing anti-Her2 isotype ADCP activity, including EC_{50} values exported directly from iQue Forecyt®.

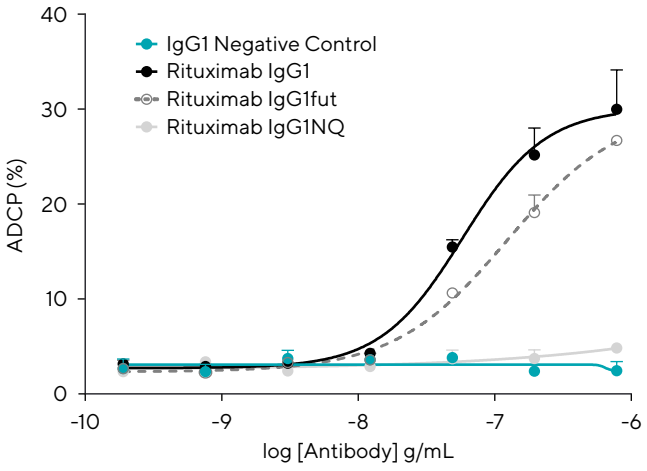
ADCP Measurement in Suspension Cells

Next, we demonstrated that the iQue® Human Antibody Dependent Cellular Phagocytosis Kit workflow could be used to quantify ADCP of CD20-positive blood cancers (Figure 5). We used the iQue® Human Antibody Dependent Cellular Phagocytosis Kit and the iQue® Platform to quantify the effect of various anti-CD20 mAbs on ADCP by PBMCs on CD20-positive Ramos target cells. The mAbs tested included three mutants of the mouse/human chimeric mAb, Rituximab: IgG1 (clinical mAb), IgG1fut (non-fucosylated) and IgG1NQ (non-glycosylated). Since antibody glycosylation is essential for Fc-receptor-mediated effector functions, the IgG1NQ mutant was not expected to induce ADCP. This is supported by the lack of ADCP response to the IgG1NQ mutant in Figure 5. As highlighted in Figure 5B, both the IgG1 and IgG1fut isotypes were expected to stimulate ADCP. The IgG-Fc mutation to remove the fucose residue was expected to enhance ADCC activity. Additionally, its effector function is similar to that of the non-mutated IgG1. We observed this with a maximal ADCP of 30% for both isotypes (Figure 5A).

Figure 5

Screening Anti-CD20 mAb Fc Mutants Revealed Differences in the Pharmacology of ADCP Induction Between Isotypes

A.



B. Rituximab Fc Mutants with EC_{50} Values and Maximal ADCP Responses

Isotype	ADCP[1]	EC_{50} (ng/mL)	Maximal ADCP (%)*
IgG1	+	59.5	30 ± 9
IgG1fut	+	126.6	30 ± 11
IgG1NQ	-	No response	No response

** Best fit top of curve*

Note. Ramos cells (2.5K/well) labeled with iQue® Proliferation and Encoding (B/Green) Dye were seeded with PBMCs (20:1 E:T) in a 384-well plate. ADCP was stimulated with a range of isotypes of anti-CD20 mAb Rituximab (0.2 ng/mL to 800 ng/mL), including: IgG1 (clinical mAb isotype), IgG1fut (non-fucosylated) and IgG1NQ (non-glycosylated). Cells were labeled using the iQue® Human Antibody Dependent Cellular Phagocytosis Kit and ADCP was analyzed using the iQue®. EC_{50} values were exported directly from iQue Forecyt®. (A) Concentration response curves for induction of ADCP in response to the mAb Rituxan and its isotypes. (B) Table describing Rituxan ADCP activity including EC_{50} values exported directly from iQue Forecyt®.

Quantification of NK Cell-Mediated ADCC

Tumor-specific induction of natural killer (NK) cell-mediated ADCC is one strategy used in developing anti-cancer mAb therapies. NK cells mediate ADCC through engagement of their FcgRIIIa (CD16a) with the constant region of antibodies bound to a target cell. This process triggers NK cell activation, including the release of proteases known as granzymes, up-regulation of Fas ligand expression, and production of cytokines, such as interferon gamma (IFNγ).

Traditional cytotoxicity assays, such as Chromium-51 (Cr-51) release or conventional flow cytometry, are time-intensive and require additional downstream assays in order to characterize donor effector cells. The iQue® Human NK Cell Killing Kit provides a multiplexed way to simultaneously assess ADCC, NK cell activation state, and effector proteins secreted in a single well of a 96- or 384-well plate, using either PBMCs or enriched NK cells as the effector source.

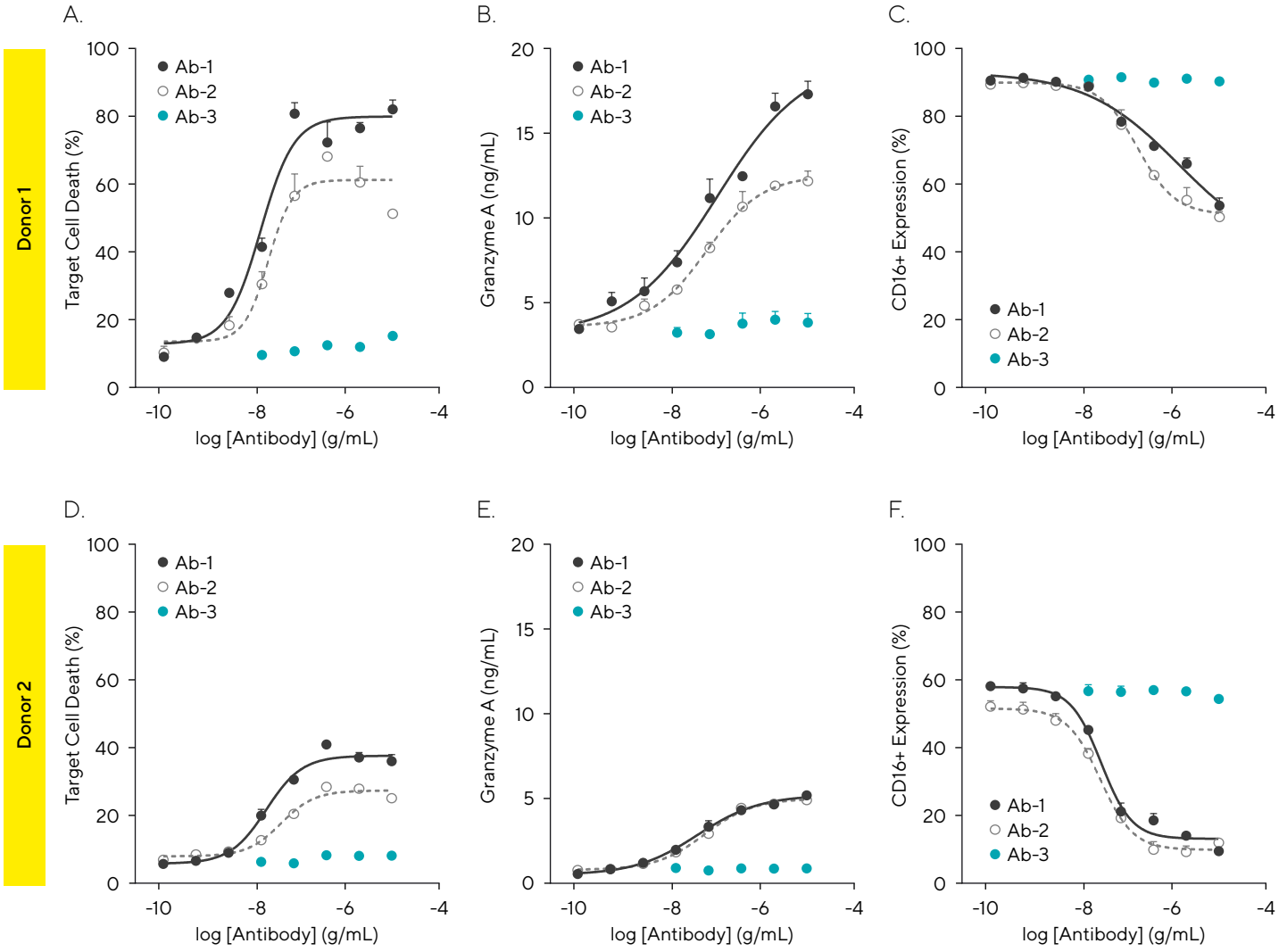
In each assay well, target cells are distinguished from effector cells by staining with a fluorescent encoder dye. Live and dead cells are separated by staining with a fluorescent membrane integrity dye that only enters dead cells or those with a compromised membrane. ADCC activity is determined by quantifying the number of dead target cells per well. Quantification of the pro-apoptotic protease, Granzyme B, is also included as another, indirect measure of NK cell cytolytic activity. Expression of CD16 is also assessed as it has been associated with ADCC activity.

Here, a co-culture assay of encoded Raji tumor cells and PBMCs from two separate donors was setup in order to measure NK-mediated ADCC and CD16 expression (Figure 6). An iQue® Human NK companion kit was also used in conjunction to quantify Granzyme A release

(Granzyme B data was comparable, data not shown). The PBMCs were incubated with one of three anti-CD20 antibodies (Ab-1, Ab-2 and Ab-3). We observed differential response to the antibodies. CD16+ expression was also negatively correlated with ADCC activity.

Figure 6

Quantify Natural Killer Cell Mediated Antibody Dependent Cellular Cytotoxicity, Cytokine/Protein Release and Marker Expression From a Co-culture Assay



Note. Encoded Raji tumor cells (20K/well) were co-cultured with PBMCs (200K/well) from two separate donors. PBMCs were incubated with one of three anti-CD20 antibodies: Ab 1 (IgG1), Ab 2 (IgG1) or a negative control Ab 3 (IgA2). Concentration range was between 10 µg/mL and 0.128 ng/mL. At 4 hours, 10 µL samples were analyzed to assess tumor cell killing using the iQue® Human NK Cell Killing Kit and the iQue® System; Granzyme A was also measured using an iQue® Human NK Cell Companion Kit. (A,D) Target cell killing by two donors show differential response to the antibodies. (B, E) Granzyme A production was both concentration- and donor-dependent. (C,F) CD16+ expression of natural killer cells decreases with increasing stimulation.

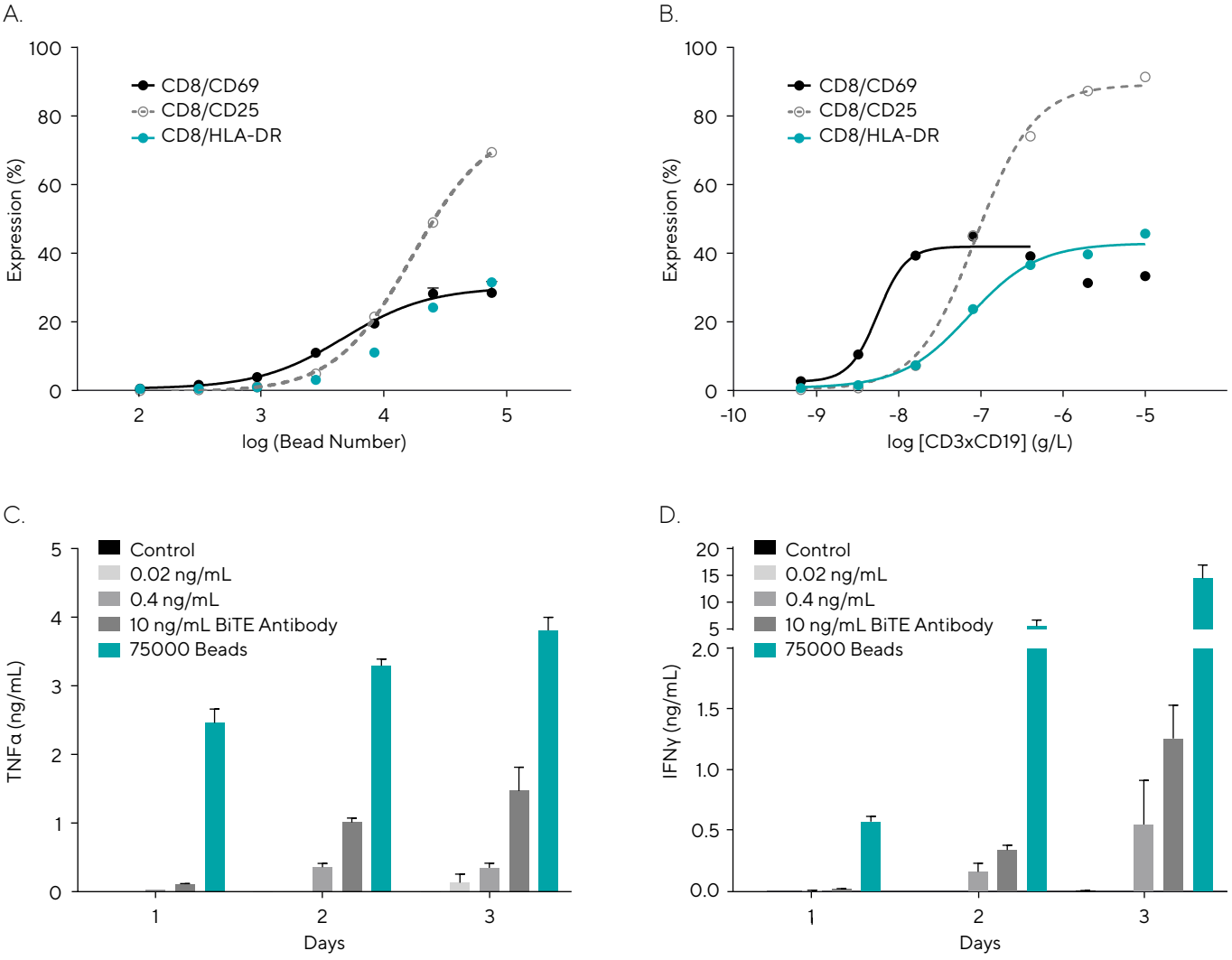
Antibody-Mediated T Cell Activation

Immunotherapies (bispecific, checkpoint inhibitor antibodies and CAR-T cells) exploit the immune system to target and eliminate cancer. For example, cytotoxicity inducers like CD3xCD19 and Herceptin block cancer's ability to escape from the immune system. Flow cytometry has played a vital role in studies of cell subtypes, activation status and cell health.

We used the iQue® Advanced Flow Cytometry Platform with a model of immune cell killing in a single plate. We activated immune cells with CD3/CD28 Dynabeads® to engage and destroy adherent and non-adherent cancerous target cells. We compared and contrasted CD3/CD28 immune cell activation to bispecific T cell engager antibody (BiTE) targeting CD3xCD19 epitopes (Figure 7).

Figure 7

Immune Cell Activation in Response to CD3xCD19 BiTE Antibody



Note. Ramos cells (15K/well) were seeded with PBMCs (1:5 Target to Effector) in a 96-well plate. Immune cell activation was induced with increasing concentrations of either CD3/CD28 Dynabeads (10^2 to 75K beads/well) or CD3xCD19 BiTE antibody (0.6 pg/mL to 10 ng/mL). Samples were analyzed using the iQue® Human T Cell Activation Kit. Daily supernatant samples (10 µL) were taken every 24 hours for cytokine analysis. Concentration response curves for T cell activation markers induced by (A) CD3xCD19 BiTE antibody or (B) CD3/CD28 Dynabeads. Analysis of cytokine concentrations (C) TNFα and (D) IFNγ comparing BiTE antibody and Dynabead activation.

In this workflow, we prepared assay plates with Ramos target cells stably expressing a nuclear targeted GFP (Incucyte® Nuclight Green), co-cultured with human PBMCs to evaluate immune cell killing of tumor cells. We added CD3/CD28 Dynabeads to induce immune cell activation. Every 24 hours, we removed supernatant and performed cytokine analysis using iQue Qbeads® (IFNγ and TNFα). Once maximal killing had been observed, cells were dissociated and evaluated for T cell subsets using the iQue® Human T Cell Activation Kit antibody panel.

The CD3/CD28 Dynabeads induced concentration-dependent increases in the proportions of CD69, CD25 and HLA-DR positive populations yielding comparable EC_{50} values (Figure 7A-B). In contrast, the BiTE antibody caused a clear left shift in the CD69 expression pattern, with low concentrations (20 pg/mL) capable of inducing almost exclusive expression of this early activation marker. Analysis of IFNγ and TNFα cytokines revealed that CD3xCD19 BiTE activation of PBMCs resulted in more effective killing (killing data not shown), despite lower cytokine release when compared to CD3/CD28 Dynabeads (Figure 7C-D).

Conclusion

Development of novel therapeutic mAbs relies on rapid identification and characterization of candidate molecules as early in the development process as possible. Traditional ELISA and flow cytometry methods require more time, use more reagent, and have limitations with respect to throughput. The high throughput iQue® Advanced Flow Cytometry Platform combined with the built-in, visual-based iQue Forecyt® software allows for assessment of multiparametric data of cell health, viability, phenotype and effector function coupled with cytokine analysis from the same well, using simple workflows and minimal sample volumes. The combination of throughput, multiparametric analysis and unique insights informs decision-making and accelerates antibody discovery workflows.

Acknowledgements

With grateful thanks to BioOutsource.

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Additional Resources

Antibody Internalization: Advanced Flow Cytometry and Live-Cell Analysis Give Rich Insights During Antibody Profiling

Quantification of Antibody-Dependent Cellular Phagocytosis via a Streamlined Advanced Flow Cytometry Workflow Application Note

Combining Live-Cell Analysis and High Throughput Flow Cytometry to Gain Additional Insights into the Mechanisms of Immune Cell Killing of Tumor Cells

North America
Sartorius Corporation
300 West Morgan Road
Ann Arbor, Michigan 48108
USA
Phone +1 734 769 1600
Email: orders.US07@sartorius.com

Europe
Sartorius UK Ltd.
Longmead Business Centre
Blenheim Road
Epsom
Surrey, KT19 9QQ
United Kingdom
Phone +44 1763 227400
Email: euorders.UK03@sartorius.com

Asia Pacific
Sartorius Japan K.K.
4th Floor, Daiwa Shinagawa North Bldg.
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Bioanalysis

Development of an immunoassay for aglycosylated murine IgG1 in mouse serum via generation of a specific tool antibody

Carrie Poon-Andersen[1], Hamsini Dhoolypala[1], Diana Wong[1], Marcus Soto[2], Hossein Salimi-Moosavi[3], Brian Chan[4], Agnieszka Kielczewska[4] & Kevin D Cook*[,1]

[1]Pharmacokinetics & Drug Metabolism, Amgen Research, 1120 Veterans Blvd, S. San Francisco, CA 94080, USA
[2]Pharmacokinetics & Drug Metabolism, Amgen Research, One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[3]Translational Safety & Bioanalytical, Amgen Research, One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[4]Biologics Discovery, 7990 Enterprise Street, Amgen Research, Burnaby, British Columbia, V5A 1V7, Canada
*Author for correspondence: Tel.: +1 650 244 2721; kcook01@amgen.com

Aim: To develop a method for the quantitation of effector functionless mouse surrogate IgG1 drug molecules in mouse matrices. **Materials & methods:** A panel of antibodies that bound specifically to N297G mutation-containing mouse IgG molecules was generated in rats. The panel was screened to identify an antibody that could be used as both the capture and detection reagent in an electrochemiluminescent immunoassay. **Results & conclusion:** The quantitative assay developed with the N297G-specific antibody passed acceptance criteria across multiple IgG1 fragment crystallizable (Fc)-containing protein formats and provides accurate quantitation of the total levels of mouse surrogate protein Fc present in *in vivo* mouse serum samples. These results are useful in understanding drug integrity and the development of precise pharmacokinetic/pharmacodynamic relationships.

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Keywords: aglycosylated antibody • generic • immunoassay • mouse IgG • pharmacokinetics • total assay

The development of protein therapeutics is dependent on robust bioanalytical methods to measure drug concentrations in biologic matrices. Accurate data from these methods is necessary to understand the pharmacokinetics (PK) and toxicokinetics (TK), as well as the development of PK/pharmacodynamic (PD) relationships and effective dose selection [1]. Ligand-binding immunoassays are the preferred method for antibody-based protein drug quantitation due to their speed, accuracy and ease of use. In clinical sample analysis, the use of anti-idiotype reagents is common [2], but due to the time and effort required for their generation, they are rarely available for discovery research PK evaluation. The most common preclinical ligand-binding assay format for antibody-based protein therapeutics is to use the target protein as a capture reagent followed by detection with a species-specific reagent. While this approach is valuable for quantitating the amount of unbound antibody that is present, it fails to detect antibodies that are in complex with a soluble form of the target or those that have lost their binding ability due to biotransformation.

The fragment crystallizable (Fc) portion of immunoglobulin (Ig) proteins has interaction sites for Fcγ effector receptors and the neonatal Fc receptor (FcRn) [3,4]. Depending on the mechanism of action of the therapeutic, it may be desired to either enhance or eliminate FcγR binding and subsequent effector function while enhancing binding to FcRn at acidic pH, which has been shown to promote FcRn-mediated recycling and extend monoclonal antibody (mAb) half-life [5,6]. Mutation of the glycosylation site Asn297 to either Ala (N297A) or Gly (N297G) results in IgG molecules with significantly reduced effector function, several of which are currently in clinical trials [7]. While the N297A mutation results in thermal instability [8], the N297G mutation gives rise to a thermally stable Fc that significantly reduces FcγR binding without impacting the manufacturing or PK properties of the molecule [9,10]. These mAbs have been named stable effector functionless (SEFL) for their preferred characteristics.

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Comparisons between assays that measure "intact" and "total" drug levels can provide valuable insight into the stability of the molecule, particularly with multispecific drug candidates that may have one or more targeting arms that are unstable [11]. Determination of total levels of human IgG molecules in preclinical species is straightforward due to the presence of highly specific antihuman Fc antibodies that can be used in a "generic" assay that measures all human IgG in the sample [12,13]. The ability to detect total levels of mouse surrogate Fc-based therapeutics is especially challenging due to the presence of endogenous mouse IgG antibodies. To circumvent this issue, the authors developed a mAb that binds specifically to N297G-mutated mouse IgG1 Fc-containing molecules. When used as either or both the capture and detection reagent in immunoassays, this mAb is a reliable tool for specific detection of multiple mouse IgG1 Fc-containing surrogate drug molecules.

Materials & methods

Materials

All biotherapeutic proteins were manufactured at Amgen, Inc. (CA, USA). Pooled mouse, rat, cynomolgus and human serum were purchased from BioIVT (NY, USA).

Immunizations

Lewis rats were immunized by hock injection with 20 μg of mouse IgG1 N297G antibody mixed with either Titermax (Sigma, MO, USA), Alum (EMD Chemicals Inc., NJ, USA), SAS (Sigma) or CpG (Eurofins MWG Operon LLC, AL, USA) 2× weekly for two months. Animals were finally boosted with protein in phosphate-buffered saline (PBS) 4 days prior to immune tissue harvest for hybridoma generation. Animals were housed under specific pathogen-free conditions at the Amgen Laboratory Research Facility and certified by the Canadian Council on Animal Care in strict accordance with associated standards and policies. The protocol was approved by the Animal Care Committee of Amgen, British Columbia.

Hybridoma generation & antigen enrichment of hybrid pools

Pooled lymphocytes obtained from spleen and draining lymph nodes were dissociated from lymphoid tissue by grinding in a suitable medium (Dulbecco's modified Eagle medium [DMEM]; Invitrogen, CA, USA). B cells were then selected and expanded using standard methods and fused with a suitable fusion partner using techniques that are known in the art [14]. Hybridoma pools were enriched for the antigen of interest by first incubating with 14 μg/ml of wild-type (WT) mouse IgG1 at 4°C for 30 min followed by the addition of 2 μg/ml biotinylated mouse IgG1 N297G antibody for an additional 30 min. Cells were then diluted 10× with 2% fetal bovine serum (FBS)/PBS (FACS buffer), spun down at 400×g for 2 min, decanted and resuspend to the original volume with FACS buffer. The biotinylated probe was detected with 5 μg/ml Alexa Fluor 647 conjugated streptavidin (Jackson ImmunoResearch, PA, USA) for 15 min, diluted 10× with FACS buffer, decanted and then resuspend in Becton Dickenson Quantum Yield (BDQY) hybridoma media (BD Biosciences, NJ, USA). Antigen-specific single cells were sorted on a FACS Aria III instrument (BD Biosciences) into 384-well plates containing BDQY hybridoma media and grown for several days in culture. The hybridoma supernatants containing monoclonal antibodies were collected and used in the screening assays to identify N297G-specific clones.

Identification of N297G-specific antibodies

Spectrally distinct xMAP LumAvidin microspheres (Luminex Corporation, TX, USA) were coated with 20 ng/ml (1.25×10^6 beads/ml) of either biotinylated mouse IgG1 N297G antibody or biotinylated WT mouse IgG1 at room temperature for 30 min in the dark. Beads were then pelleted at 1500×g for 2 min, washed 2× with FACS buffer and then resuspended with StabilGuard (Surmodics, MN, USA). Hybridoma supernatants were screened by adding 2500 beads of each coated bead set, incubating for 1 h, washing 2× with FACS buffer and detecting bound antibody with 5 μg/ml secondary antibody mouse-anti-rat Alexa Fluor 488 (Jackson ImmunoResearch). Resolution of bead populations and secondary antibody binding were then analyzed by flow cytometry on the iQue Screener system (Sartorius, Göttingen, Germany).

Screening of N297G-specific antibodies

The screening of the panel of rat anti-mouse N297G IgG1 antibodies was performed via ELISA. The standard samples of a mouse N297G IgG1 antibody specific to the target protein A were prepared in buffer and serum from mouse, rat, cyno or human serum. ELISA plates were coated with the specific anti-mouse N297G antibody or a

nonspecific goat anti-mouse (H+L) polyclonal antibody (Jackson ImmunoResearch). The plate was then washed and blocked with I-Block™ (Life Technologies, CA, USA) buffer. The standard samples were pretreated in assay buffer (I-Block™ +5% bovine serum albumin [BSA]) 1:10 prior to the assay. The pretreated samples were added to the plate, incubated for 1 h and then washed. Either of the following detection strategies was then performed: biotinylated anti-mouse N297G antibody followed by horseradish peroxidase (HRP)-conjugated streptavidin, biotinylated target A protein followed with HRP-conjugated streptavidin or HRP-conjugated nonspecific goat anti-mouse (H+L) polyclonal antibody (Jackson ImmunoResearch) antibody. After 1 h incubation with the detection reagent, the ELISA plate was washed and the One component 3,3',5,5' tetramethylbenzidine dihydrochloride (TMB) substrate (Seracare, MA, USA) was added to the plate and the color development was measured by ELISA Kinetic at 650 nm using a SpectraMax plate reader (Molecular Devices, CA, USA).

Mouse pharmacokinetic studies

Female WT C57L/6J were purchased from Jackson Laboratory (MA, USA). C57BL/6N mice expressing a chimeric mouse/human CD3ϵ receptor used for bispecific T-cell engager (BiTE) protein studies were maintained at Charles River Laboratories [15]. The proteins of interest were administered as a 1 mg/kg or 10 mg/kg intravenous bolus dose via the lateral tail vein. Blood specimens were collected at various times post-injection, incubated at ambient temperature for approximately 20 min or until fully clotted and then centrifuged to separate out the serum. All serum specimens were stored at -70°C (±10°C) until use in analytical assays. Mice were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals, 8th Edition* at AAALAC, International accredited facilities. All mice protocols were approved by the Amgen, Inc. Institutional Animal Care and Use Committee (CA, USA).

Pharmacokinetic sample analysis

Quantitation of proteins in mouse serum was performed using electrochemiluminescent immunoassays on the MSD Sector 600 instrument (Meso Scale Diagnostics, MD, USA). For the total N297G assay, biotinylated anti-mouse N297G antibody coated onto Streptavidin SECTOR plates was the capture reagent. The standards and samples were pretreated in assay buffer (Blocker™ BLOTTO in Tris-buffered saline [TBS]) 1:20 prior to the assay. The pretreated samples were added to the plate and incubated for 1 h, followed by washing and incubation with a ruthenylated anti-mouse N297G antibody as the detection reagent. For the intact assays, the plates were instead coated with biotinylated target protein #1, #2, #3 or biotinylated anti-cytokine antibody as the capture reagents, respectively, with a ruthenylated anti-mouse IgG, Fcγ subclass 1 specific (Jackson ImmunoResearch) antibody as the detection reagent. In all assays, the analyte serum concentrations were interpolated from standard curves using the corresponding analyte prepared in pooled mouse serum.

Results

Quantitation of mouse surrogate IgG drug molecules in mouse matrices (serum, plasma, etc.) is limited by the reactivity of anti-mouse IgG reagents with the endogenous antibodies present. As such, all immunoassay methods to detect these molecules rely on the presence of at least one specific reagent, typically the target protein, which is used as a capture reagent paired with an anti-mouse IgG detection antibody. To circumvent this, the authors endeavored to generate a reagent that binds specifically to effector functionless mouse surrogate drug IgG molecules and could be used interchangeably across multiple molecules, utilizing the N297G mutation for specificity. Hybridomas were generated from Lewis rats immunized with an N297G-containing mAb and their supernatants were screened for binding to N297G mouse IgG1 antibodies. A panel of 11 rat anti-mouse N297G antibodies was selected for further analysis by direct ELISA using plates coated with a WT or N297G mouse antibody followed by detection with the biotinylated rat anti-mouse N297G antibodies followed by Streptavidin-HRP. There were seven clones in the panel that were specific to only the mouse N297G antibody without binding to the WT mouse antibody (data not shown). To select the best candidate, additional experiments were conducted using the panel of antibodies as either a capture or detection reagent paired with specific or nonspecific reagents. For simplicity, the data presented here are only for the rat anti-mouse N297G antibody (clone 1C5) that was ultimately selected as the best molecule. Standard curves of a mouse N297G antibody were prepared in either buffer or serum from multiple species and tested in various assays. The first test involved using the anti-mouse N297G antibody as a capture reagent paired with a nonspecific anti-mouse detection antibody. ELISA analysis of the standard curves prepared in buffer and non-mouse serum yielded sufficient data with a broad dynamic range (Figure 1A). However, in mouse serum, there was a very high background signal observed, likely due to the nonspecific anti-mouse detection antibody

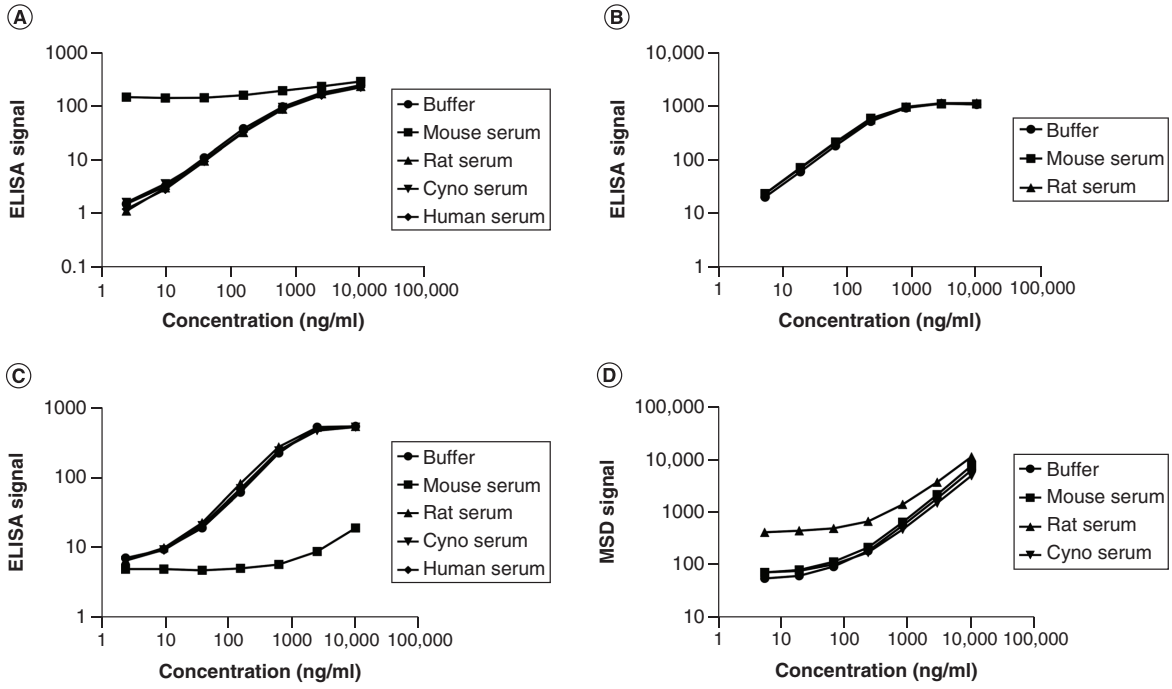

Figure 1. Screening of anti-mouse N297G antibody as capture or detection antibody. Standard curves of an N297G mouse IgG1 were prepared in the indicated matrices and detected via the following assay formats: **(A)** ELISA using anti-mouse N297G for capture with polyclonal anti-mouse as detection; **(B)** ELISA using anti-mouse N297G for capture with specific analyte as detection; **(C)** ELISA using polyclonal anti-mouse for capture with anti-mouse N297G as detection; and **(D)** electrochemiluminescent assay using antimouse N297G for capture and detection. In each graph, the data are presented as the mean of n ≥ 2.

(Figure 1A). In the second test, the anti-mouse N297G antibody was also used as a capture reagent but a specific reagent was instead used as the detection reagent. In this case, the standard curves were identical in all matrices, as the specificity of the detection reagent greatly reduced the background in mouse serum (Figure 1B). In the third test, the orientation of the first test was reversed and the anti-mouse N297G antibody was used for detection combined with a nonspecific anti-mouse capture antibody. Similar to the first test, satisfactory standard curves were observed in buffer and non-mouse serum (Figure 1C). In mouse serum, the signal was much lower than in the other matrices due to the nonspecific capture reagent binding to the endogenous mouse IgG present in mouse serum (Figure 1C). Despite the poor performance of these assays in mouse serum, the results thus far indicated that the anti-mouse N297G antibody had potential as a capture and detection reagent. To determine if this antibody could be used as both a capture and detection reagent in the same assay, the standard curves were tested in an MSD electrochemiluminescent assay. The standard curves were indistinguishable in mouse serum, cyno serum and buffer, though the background and overall signal were slightly higher in rat serum, suggesting there may be a low level of cross-reactivity of the anti-mouse N297G antibody with rat IgG (Figure 1D). Taken together, these data indicate that this antibody can be used in immunoassays for quantitation of N297G-containing mouse surrogate IgG in mouse serum as either a capture or detection reagent, or as both the capture and detection reagent in the same assay.

The ability of the anti-mouse N297G mAb to be used as both the capture and detection reagent in the same assay suggests that it could be used as a "generic" assay across any molecules containing a mouse Fc with the N297G mutation. To test this, the assay was performed on the standard curve and quality control (QC) samples of four unique mouse surrogate protein therapeutics in various molecular formats including mAb, BiTE protein, antibody–cytokine fusion (Ab-cyt) and bispecific antibody (bsAb). The assay precision and accuracy of the calibrator standard and QC samples were monitored across four independent measurements for each protein prepared in pooled mouse serum. The assay performed well with a nearly 3-log dynamic range of quantitation between the upper and lower limits of 10,000 and 39.1 ng/ml, respectively. The bias and coefficient of variation (%CV) were within the general acceptance criteria of 20% for all samples, except for the lowest standard point and QC samples

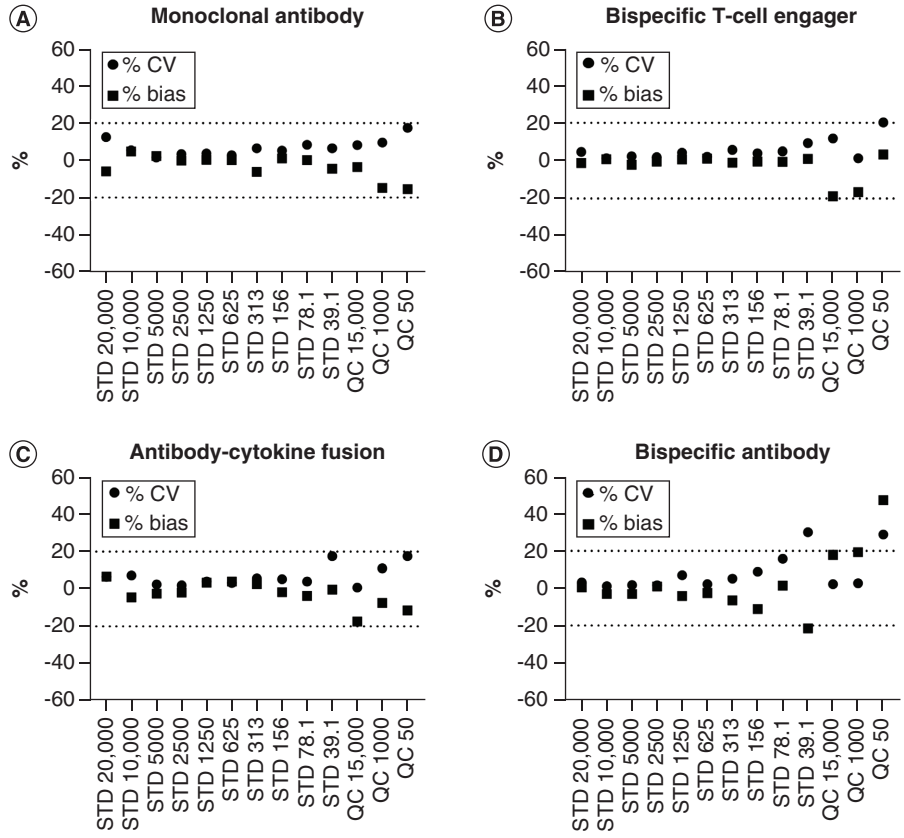

Figure 2. Inter-assay precision and accuracy from the standard curve and quality control samples in mouse serum. Standard curve and quality control samples of four N297G mouse IgG1-containing proteins were prepared in mouse serum and detected via an electrochemiluminescent assay using anti-mouse N297G for capture and detection. The inter-assay precision (%CV) and precision (%bias) are presented for the four unique proteins as follows: **(A)** monoclonal antibody; **(B)** bispecific T-cell engager; **(C)** antibody–cytokine fusion; and **(D)** bispecific antibody. The precision and accuracy were calculated from n = 4–6 replicates per data point.

for the bispecific antibody (Figure 2). These data show that this assay can be used interchangeably across a variety of protein structures for the detection of mouse surrogate drug molecules in mouse serum samples.

The utility of the generic anti-mouse N297G assay was tested by measuring sample concentrations from PK studies of each of the four different molecules that were tested in comparison with their measurement by "intact" immunoassays. The mAb concentrations were similar between both assays, with the intact assay referring to the target protein used as a capture combined with an anti-mouse Fc detection antibody (Figure 3A). In contrast, the BiTE protein resulted in much higher concentrations for the total assay compared with the intact assay, which was determined with a target protein capture/anti-mouse Fc detection assay format (Figure 3B). The target used for this assay was not the T-cell targeting CD3, but rather the intended target for lysis on tumor cells. To further validate the accuracy of the measurements performed with this assay, the concentrations of a different BiTE protein were measured in a PK study with an orthogonal liquid chromatography–mass spectrometry (LC-MS) assay. The concentrations from the two methods performed in parallel yielded indistinguishable results, highlighting the ability of the immunoassay presented here to accurately quantitate agylcosylated murine antibodies in mouse serum (data not shown). The Ab-cyt protein intact assay (anti-cytokine capture/anti-mouse Fc detection) concentrations were significantly lower than the total assay, similar to the BiTE protein analysis (Figure 3C). The total and intact (protein capture/anti-mouse Fc detection) assay measurements were equivalent for the bsAb (Figure 3D). These data collectively show that the N297G assay provides valuable quantitative data on the total amount of therapeutic protein Fc present in *in vivo* mouse samples, which can often be drastically different than measurements from intact assay formats.

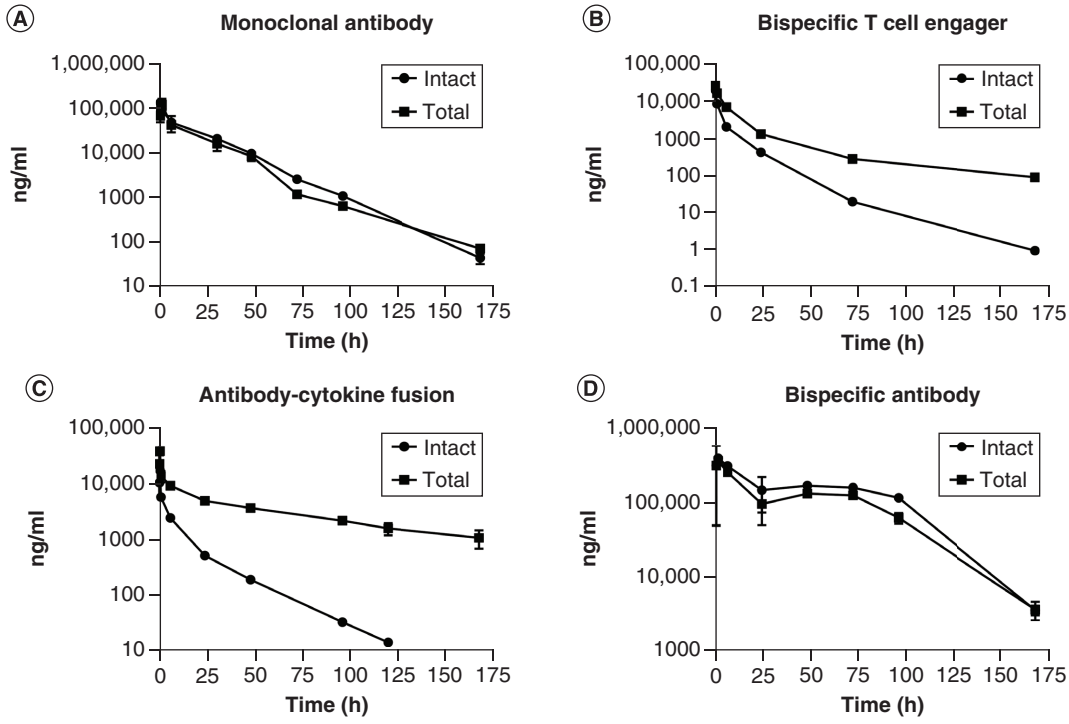

Figure 3. Serum time-concentration pharmacokinetic profiles of various proteins measured via intact/total assay formats. The concentrations of four unique analytes were determined in mouse serum after being injected intravenously into C57BL/6J mice and measured by total (N297G capture and detection) or intact assay formats. **(A)** Monoclonal antibody. **(B)** Bispecific T-cell engager. **(C)** Antibody–cytokine fusion. **(D)** Bispecific antibody. Each data point is presented as mean ± standard deviation; n = 3 mice per data point.

Discussion

Herein, the authors describe the generation of an anti-mouse IgG antibody that specifically recognizes aglycosylated N297G-mutated Fc proteins. Using this antibody, an electrochemiluminescent immunoassay was developed for the quantitation of mouse surrogate IgG Fc that works in mouse serum despite the presence of high levels of endogenous mouse IgG. The assay performance for calibrator standard curves met acceptance criteria (<20%) for precision (%CV) and accuracy (%bias) across a wide dynamic range. This assay was implemented to interchangeably measure *in vivo* PK samples from a variety of IgG Fc-containing mouse surrogate protein structures. The results generated with the total measurement described here can be considerably different than intact assay measurements.

Translation of preclinical PK/PD relationships from animal models to human predictions is crucial for protein therapeutic development. When the human therapeutic molecule does not cross-react with rodent proteins, these relationships are built with the use of surrogate molecules, often performed in mice. The accuracy of these models is highly dependent on precise bioanalytical measurement of mouse surrogate molecules in mouse samples, which can be complicated by the presence of endogenous mouse IgG proteins. Ligand-binding assays are the preferred method for the generation of bioanalytical PK sample data due to their sensitivity and throughput, but they are entirely dependent on the quality of the reagents available for use. As the speed of drug development continues to accelerate, the amount of time allotted for bioanalytical method development is frequently reduced. The assay and antibody described here are valuable because they can be used for any effector functionless N297G mouse IgG1 molecule as either the capture or detection reagent, or simultaneously as both the capture and detection reagents. This allows for rapid method development timelines with minimal effort needed to transfer between different molecules.

The generation of analytical reagents specific to the drug molecule of interest is the gold standard for ligand-binding assays. For antibody-based therapeutics, these reagents are primarily anti-idiotype antibodies that bind to the complementarity-determining region of the protein. However, these antibodies take a significant amount of time and effort to generate and are rarely developed for surrogate molecules. In the absence of anti-idiotype reagents, the target protein is commonly used as a capture reagent. However, in some instances, the recombinant form of

the target protein is difficult to produce (i.e., multimembrane-spanning receptors) or not available/economically challenging to obtain commercially. One potential alternative for data collection in this scenario involves labeling the surrogate drug protein prior to injection; however, avoiding any potential impacts of labeling on biodistribution and PK is preferred [16]. The assay described here is especially useful when the target protein is challenging, as the dependence on sourcing difficult-to-obtain reagents and/or introducing an artificial label is eliminated.

While the assay described here is focused on a ligand-binding assay using the anti-mouse N297G antibody, this reagent has the potential to be used in many different analytical methods. LC-MS assays often rely on an immunoprecipitation step prior to analysis and the ability to specifically capture mouse surrogate molecules can be applied to this technology [17]. Additionally, *ex vivo* microscopy-based imaging techniques on mouse tissue samples could also be aided by the specificity of the antibody generated here. These approaches are outside the scope of this manuscript, but the authors are actively pursuing them for various projects.

Conclusion

To our knowledge, this is the first description of an antibody that specifically recognizes N297G-mutated mouse surrogate IgG molecules. The quantitative assay developed with this antibody is suitable for the detection of multiple IgG1 Fc-containing protein formats. The ability to reliably quantitate the level of mouse surrogate protein Fc in mouse serum samples is a beneficial tool for the rapid development of protein therapeutic candidates.

Future perspective

As the speed at which drug discovery rapidly accelerates due to advances in technology and increased competition, the utility of generic assays that can be used across multiple programs will be prioritized. The ability to use the same assay reagents is critical for reducing method development time and ensuring timely delivery of PK data. The authors hope that in the future, the method presented herein and others similar to it will be widely used for rapid quantitation of protein-based therapeutics.

Summary points

- Quantitation of mouse surrogate IgG1 drug molecules in mouse matrices can be challenging due to the background from endogenous mouse IgG.
- A novel N297G-specific rat anti-mouse IgG1 antibody was developed to bind specifically to aglycosylated mouse IgG1 proteins.
- An immunoassay using this antibody was established with acceptable assay precision and specificity.
- This assay can be used across multiple mouse surrogate IgG1 molecules for quantitation of the total drug level present in mouse matrices.

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Financial & competing interests disclosure

All authors were employed by Amgen, Inc. at the time the work was conducted. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The rat study protocol was approved by the Animal Care Committee of Amgen British Columbia. All mice protocols were approved by Amgen, Inc. Thousand Oaks Institutional Animal Care and Use Committee.

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- **Provides a synopsis of the importance of the detection of free and total protein therapeutic concentrations.**

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- **This work shows a comparable method for the quantitation of total levels of human IgG.**

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BioTechniques Spotlight

SARTORIUS

Key trends in antibody discovery

For our Spotlight on antibody discovery, we surveyed our audience to find out the latest trends, opinions and behaviors in the field.

Your work

What therapeutic area(s) do you work in?

Over half the people surveyed worked in cancer research.

- Cancer **58%**
- Immunotherapy **45%**
- Autoimmune diseases **21%**
- Inflammation **24%**
- Infectious diseases (inc. COVID-19) **36%**
- Neurodegenerative **18%**
- Cardiovascular **9%**
- Rare diseases **18%**
- Metabolic **12%**
- Ocular **6%**
- Regenerative medicine **12%**
- Drug-resistant bacteria **3%**
- I don't work in a therapeutic area **6%**

What type of therapeutics do you develop?

- Protein therapeutics **40%**
- Monoclonal antibodies **42%**
- Bispecific antibodies **15%**
- Trispecific antibodies **3%**
- Antibody–drug conjugates **27%**
- CAR-T/cell therapies **21%**
- I don't develop therapeutics **24%**

What type of cells do you generally use as a source?

- Primary human immune cells 52%
- Other species immune cells 30%
- Non-adhered cell lines 15%
- Adhered cell lines 27%
- iPSC or iPSC-derived cells 15%
- Bacterial or fungal cells 27%

Discovery technologies

What technique(s) do you use for antibody discovery?

Hybridoma and surface display technologies were the most popular.

- Hybridoma technology 48%
- Surface display 42%
- Primary B-cell cultures 33%
- Direct screening 27%
- Transgenic technology 18%

How early in your discovery pathway do you assess functional activity of your antibodies?

- Hit generation (100-1000 samples) 26%
- Lead characterization (10-50 stage) 26%
- Candidate selection (1-10 stage) 48%

What functional activities of your test antibody are of most interest to you?

- Binding affinity/potency 78%
- Antibody-dependent cellular cytotoxicity 50%
- Antibody-dependent cellular phagocytosis 28%
- Internalization 28%

What bioassay(s) do you use to quantify antibody activity?

- ELISA 72%
- Kinetic protein–protein interactions 27%
- Reporter – fluorescence or luminescence 36%
- Flow cytometry 55%

The people who used flow cytometry ranked their most important features of a flow cytometry assay for antibody activity as:

1. Speed and throughput
2. Volume of sample required
3. Ability to multiplex outputs
4. Integrated analysis software
5. Cost of analysis

Do you incorporate artificial intelligence/machine learning into your antibody discovery efforts?

Only 25% of people incorporate AI/ML into their discovery efforts, with the main barrier being training.

YES 25%

NO 75%

What are the main barriers to this?

- **Training** – I don't know where to start or how it could help me **40%**
- **Hardware** – I can't manage this on my machine **12%**
- **Not relevant to me** **16%**
- **Software** – I can't find any software that performs the type of analysis I need **32%**

Challenges

Do you think reproducibility is an issue in the field?

Yes **84%**
No **16%**

The majority of respondents thought reproducibility was an issue in antibody discovery.

What is the main challenge faced in antibody discovery?

What is the biggest technology gap or need in antibody discovery?

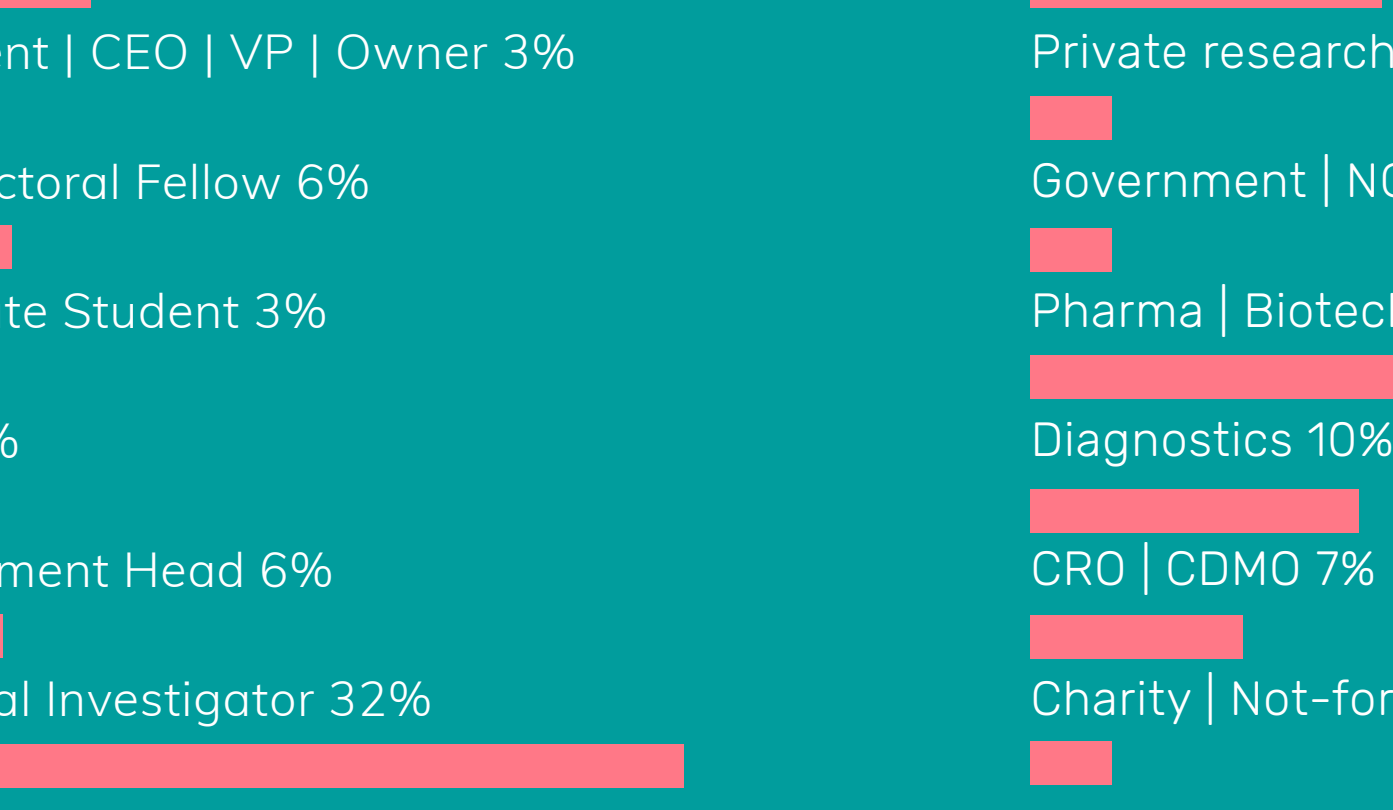
- Tools for characterization and target identification
- Antibody versus target sequence/modeling
- Lack of available antibodies for research needs
- Tools for efficacy testing and prediction
- Reliable reagents
- ?

What excites you most about the future of antibody therapeutics?

- The potential for using machine learning in immunotherapy
- Ability to target rare disorders
- Targeted therapies
- Their efficiency and positive effects on patient survival
- Their use in infectious diseases

About the respondents

Location



Job role

- Staff Scientist 14%
- Lab Director | Chief Scientist 9%
- President | CEO | VP | Owner 3%
- Postdoctoral Fellow 6%
- Graduate Student 3%
- PhD 3%
- Department Head 6%
- Principal Investigator 32%
- Professor | Instructor 9%
- Medical Profession | Physician 6%

Sector:

- Academic 45%
- Hospital | Clinic 13%
- Private research Foundation 3%
- Government | NGO 3%
- Pharma | Biotech 16%
- Diagnostics 10%
- CRO | CDMO 7%
- Charity | Not-for-profit 3%

BioTechniques Spotlight

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Application Note

Keywords or phrases:
Antibody discovery, live-cell antibody binding, monoclonal antibodies, antibody:antigen interactions, iQue®, advanced flow cytometry, iQue Forecyt® software

Utilizing Advanced High-Throughput Flow Cytometry to Quantify Direct and Competitive Live Cell Antibody Binding

Kirsty McBain, Daryl Cole and Nicola Bevan
Sartorius UK Ltd., Units 2 & 3 The Quadrant, Newark Close, Royston, Hertfordshire, SG8 5HL, UK

Introduction

Monoclonal antibody (mAb) therapeutics comprise one of the most rapidly growing drug classes, with the milestone for the 100th FDA approval of a mAb product reached in 2021[1,2]. Amongst these approved products are treatments against a huge range of diseases, namely, a large number of cancer therapeutics, comprising about 45% of approved mAbs, and interventions against immune disorders, comprising about 27% of products[3]. Within these categories are mAbs raised against a wide variety of target antigens, for example anti-HER2 (human epidermal growth factor receptor 2) mAbs to target breast cancers and anti-TNF (tumor necrosis factor) antibodies to treat symptoms of inflammatory disease[3].

The success of mAbs as a class of therapeutics is largely due to their high specificity and affinity for the target antigen. Evaluation of mAb target binding is a critical part of the antibody development process and robust, high-throughput techniques are needed to facilitate rapid screening of antibody libraries. Utilizing such techniques as early as possible in the drug discovery pathway can enhance the quality of 'hits' generated for mAbs with desirable characteristics, providing increased likelihood of candidates being progressed through the clinical pipeline.

Specific binding of antibody to the target antigen is one of many key attributes that must be evaluated during development, alongside characteristics such as Fc function, stability and post-translational modifications. Conventional techniques for evaluating antibody binding can often:

- Have low-throughput and long sample acquisition times
- Measure binding to purified, truncated or tagged recombinant forms of the target protein
- Be laborious and time-consuming, requiring steps such as protocol optimization, fixation and repetitive washes
- Necessitate the use of large volumes of precious sample and antibody.

Here we present two simple assays that utilize the iQue® Advanced Flow Cytometry Platform and validated reagents to measure binding of unlabeled therapeutic mAbs to your target on live cells. The first is a direct antibody binding assay which ranks mAbs based on binding to target cells, with the ability to analyze binding to multiple cell types in a single well. The second is a competitive binding assay which can reveal mAbs that target different epitopes.

Method and Workflow

Direct binding assay

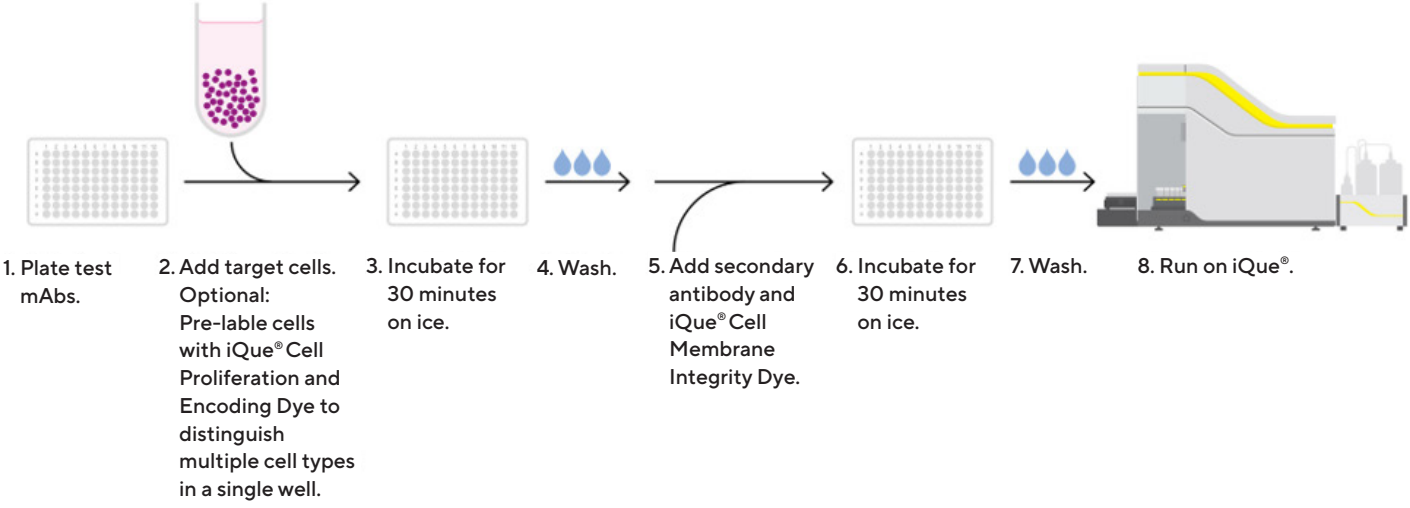


Figure 1: *Direct Antibody Binding iQue® Assay*

Note: Simple workflow to assess binding of test mAbs to live cells using the iQue® Advanced Flow Cytometry Platform. Target cells are optionally labeled with iQue® Cell Proliferation and Encoding (V/Blue) Dye and incubated with test mAbs for 30 minutes on ice. Plates are then washed and labeled with iQue® Cell Membrane Integrity (R/Red) Dye. After 30 minutes incubation, plates are washed and run on the iQue®.

1. Dilute test mAbs to desired concentration in cell culture media at 3× final assay concentration (FAC). Add 10 µL/well to a 96- or 384-well V-bottom plate (e.g., Corning 3363 or 3656).
2. Optional: If adding multiple cell types per well, distinguish cells by labeling with iQue® Cell Proliferation and Encoding (V/Blue) Dye:
   I. Prepare a working stock of encoder dye by diluting in PBS (dilution factor e.g., 1 in 1000 for bright staining).
      - Note: If using more than two cell types per well, the bright dye can be diluted (e.g., 1 in 10) to create dimmer populations.
   II. Collect cells in a conical tube, wash in PBS and resuspend at 2×10^6 cells/mL.
   III. Add an equal volume of the prepared dye solution (1:1) and incubate (37 °C, 15 minutes).
   IV. Wash cells with at least 2× volume of cell culture media (including 10% serum), spin (500 g, 5 minutes).
   V. Repeat wash step twice more.

3. Prepare target cells to an appropriate density in cell culture media and add 20 µL/well to the plate. Incubate for 30 minutes on ice.
   - Note: Recommend a starting cell density of approximately 5–10 K cells/well for each cell type.
4. Add 100 µL of wash buffer (e.g., PBS + 2% FBS) and centrifuge (300 g, 5 minutes).
5. Remove media and shake (2000 RPM, 1 minute) using the iQue® plate shaker to resuspend cells.
6. Combine fluorophore conjugated secondary antibody (e.g., R-Phycoerythrin-conjugated AffiniPure F(ab')2 Fragment Goat Anti-Human IgG, Jackson ImmunoResearch) with iQue® Cell Membrane Integrity Dye (choice of V/Blue (if not using V/Blue encoder dye), B/Green, B/Red, R/Red) at the concentration recommended in the dye protocol. Add 10 µL/well of the mixture to the plate and incubate for 30 minutes on ice.
7. Wash, spin and resuspend as in steps 3–5. Add 20 µL/well wash buffer.
8. Run on the iQue® using a 4 and 5 second sip time and analyze median fluorescence intensity (MFI) using iQue® Forecyt® software.

Competitive binding assay

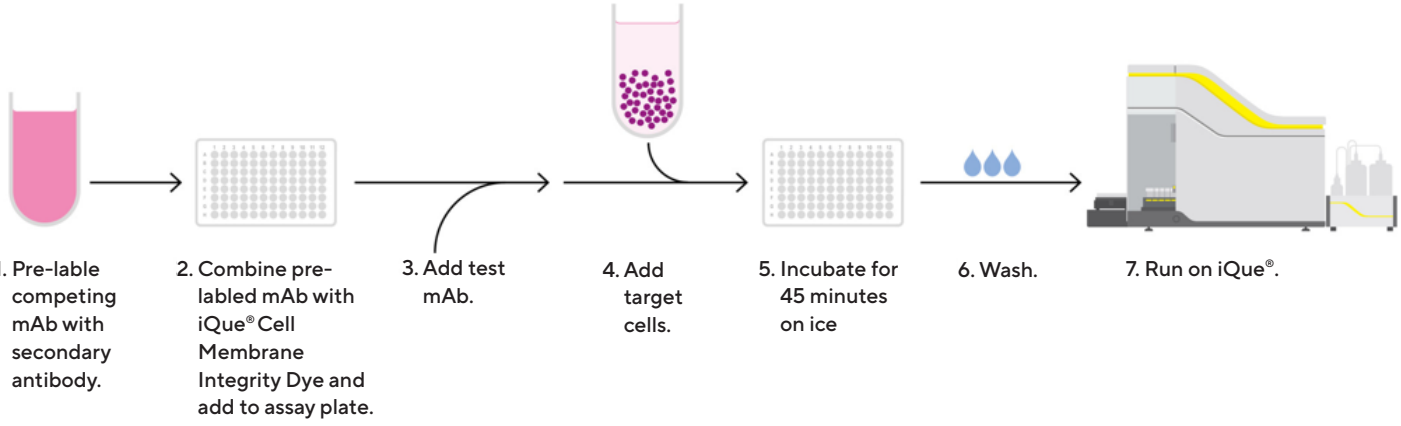


Figure 2: *iQue® Competition Binding Assay Workflow*

Note: A competing antibody is pre-labeled in a tube with a fluorophore-conjugated secondary antibody, prior to combination with the unlabeled test antibody of interest in the assay plate. The target cells are then added to this antibody mixture and the labeled competing mAb and the test mAb will compete for binding to the target antigen, provided they both target the same epitope. Therefore, the greater the concentration of the test antibody, the less of the labeled competing antibody will bind, resulting in a lower MFI value for the secondary antibody fluorophore. If the two antibodies don't compete for the same epitope, the MFI should be unaffected by test mAb concentration.

1. Pre-label test mAb (3×FAC) in a tube with fluorophore conjugated secondary antibody at a 1:1 concentration ratio for 15 minutes at room temperature.
2. Combine with iQue® Cell Membrane Integrity Dye and add 10 µL/well of the mixture to a 96- or 384-well plate.
3. Dilute unlabeled test competing mAbs to desired concentration and add to the assay plate (10 µL/well).
4. Prepare target cells to an appropriate density in cell culture media and add 10 µL/well to the plate. Incubate for 45 minutes on ice.
   - Note: Recommend a starting cell density of approximately 10–20 K/well for each cell type.
5. Add 100 µL of wash buffer (e.g., PBS +2% FBS) and centrifuge (300 g, 5 minutes).
6. Remove media and shake (2000 RPM, 1 minute) using the iQue® plate shaker to resuspend cells.
7. Add 20 µL/well wash buffer.
8. Run on the iQue® using a 3–5 second sip time and analyze MFI using iQue Forecyt® software.

Results

Assessment of mAb binding and specificity in both adherent and suspension cell models

Historically, many flow cytometry assays focused more on analyzing cells grown in suspension culture and less on adherent cell types, largely due to issues with lifting and handling the cells. This presents a huge limitation in the field of oncology, since around 90% of adult cancers are solid tumors formed from adherent cell types[4,5]. To expand the use of the iQue® antibody binding assay, we validated it for use with both suspension and adherent cell types.

For the suspension cell model, we compared binding of anti-CD20 antibody Rituximab to CD20-antigen positive Ramos cells, from a B lymphocyte cell line, and CD20-negative Jurkat cells, from a T lymphocyte line. For the adherent cell model, binding of anti-HER2 antibody Trastuzumab was quantified on HER2-positive AU565 cells and HER2-negative MDA-MB-468 cells, both from breast cancer cell lines. In both models, the antigen negative cell type was labeled using the iQue® Cell Proliferation and Encoder (V/Blue) Dye to distinguish them from the antigen positive cells, as displayed in the dot plots in Figure 3.

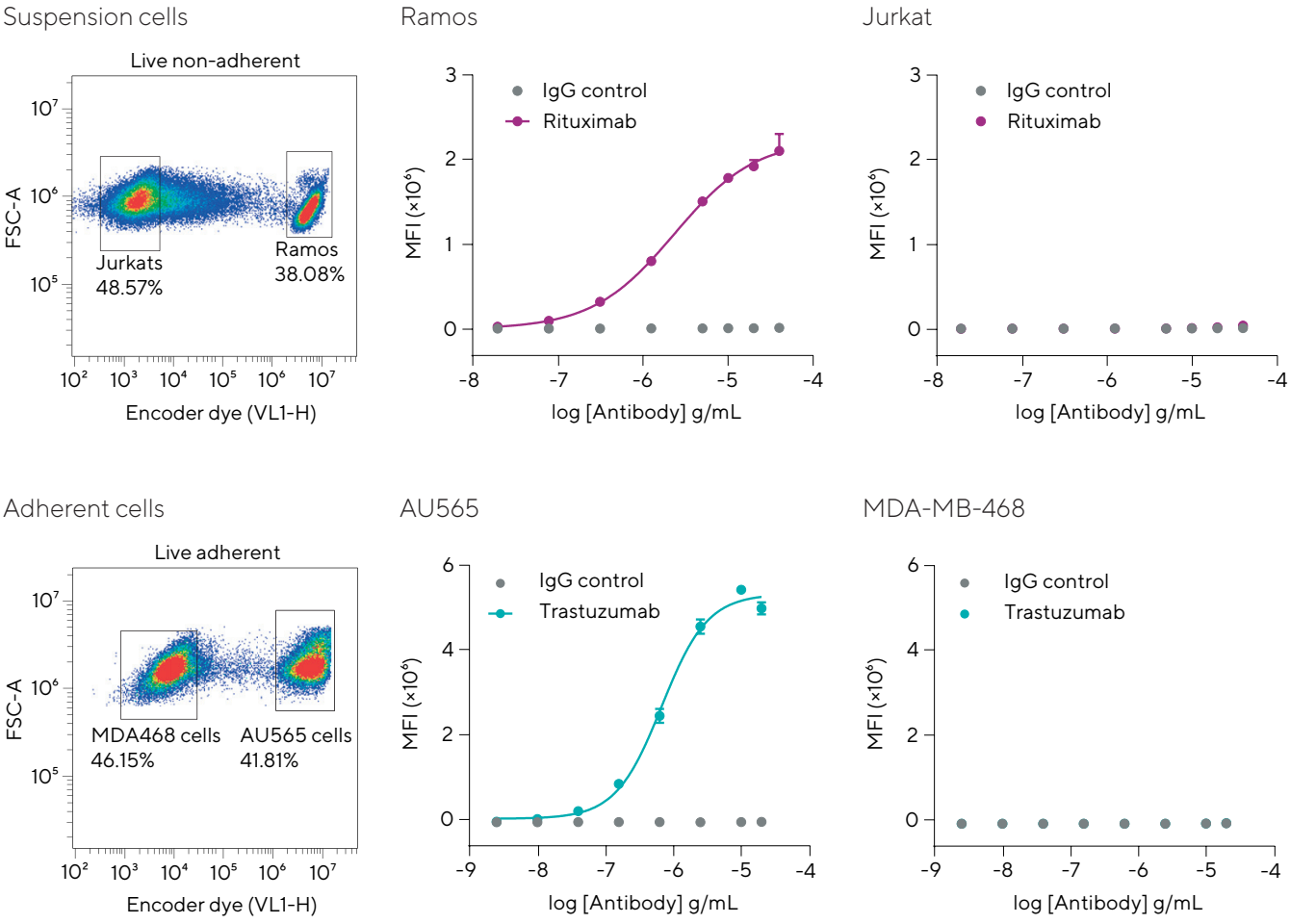


Figure 3: *Assess Specificity of Antibody Binding to Target Antigens on Both Suspension and Adherent Cells*

Note: Binding and specificity of two therapeutic antibodies, Rituximab and a Trastuzumab biosimilar, was assessed using a suspension and an adherent cancer cell line model. CD20-positive Ramos cells and HER2-positive AU565 cells were labeled with iQue® Cell Proliferation and Encoder (V/Blue) Dye to distinguish them from antigen negative Jurkat and MDA-MB-468 cells. Cells were incubated with a range of concentrations of unlabeled test mAb or an IgG control followed by a single concentration of RPE-conjugated secondary antibody (5 µg/mL).

In both the suspension and adherent models, there was an antigen positive cell type-specific increase in binding with increasing antibody concentration. There was no binding observed with the antigen negative cell types, or with the IgG control antibody.

It is crucial to assess whether there is any off-target binding of a novel antibody candidate in the early stages of drug discovery using *in vitro* assays, as this can present issues such as reduced efficacy and increased toxicity *in vivo*[6].

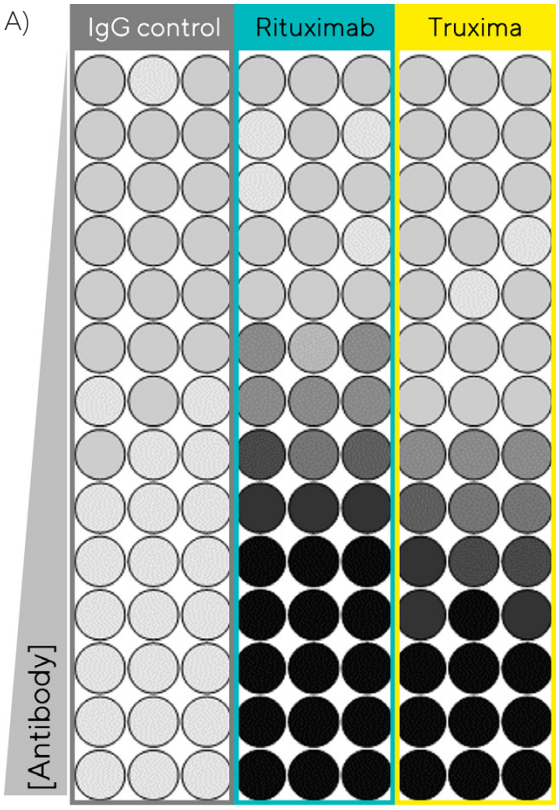
4

Comparing biosimilar binding to live target cells

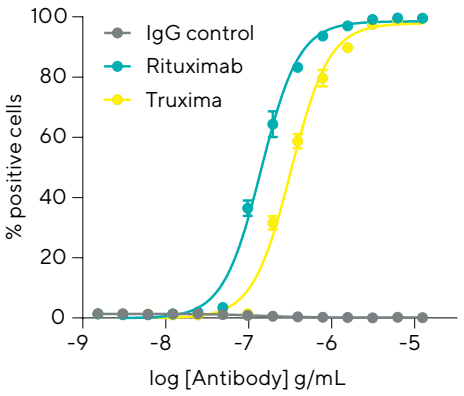
mAb biosimilars have been a relatively recent emergence on the bio-therapeutic market and have the potential to encourage lower cost treatments and to increase access for patients. For a novel mAb biosimilar to be approved for use, characteristics such as antigen binding, Fc receptor interactions and Fc function must be rigorously tested to show that they are comparable to the reference product. Here we used the iQue® direct binding assay to compare the binding of anti-CD20-IgG1 antibody Rituximab to one of its biosimilars, Truxima (Figure 4). Although there was a concentration dependent increase in binding of both antibodies to the Raji target cells, there was a clear shift to indicate decreased potency binding of the Truxima compared to Rituximab, with EC_{50} values of 0.14 ± 0.01 µg/mL and 0.32 ± 0.03 µg/mL for Rituximab and Truxima, respectively.

This difference between Rituximab biosimilars has been seen before, for example, our previous work showed there was a difference in the level of ADCP induced by Rituximab and Truxima. Other studies have shown differences in ADCC and CDC activity between Rituximab and its biosimilars[7,8]. There are several possible reasons for these differences in Fc function between biosimilars, for example, their levels of post-translational modifications could differ, which depends on factors such as the expression system, or culture conditions used[8,9].

A)



B)



C)

Compound	Top	EC_{50} ± 95% CI (µg/mL)	Slope	R^2
Rituximab	98.6	0.14 ± 0.01	1.8	0.995
Truxima	98.0	0.32 ± 0.03	1.9	0.994

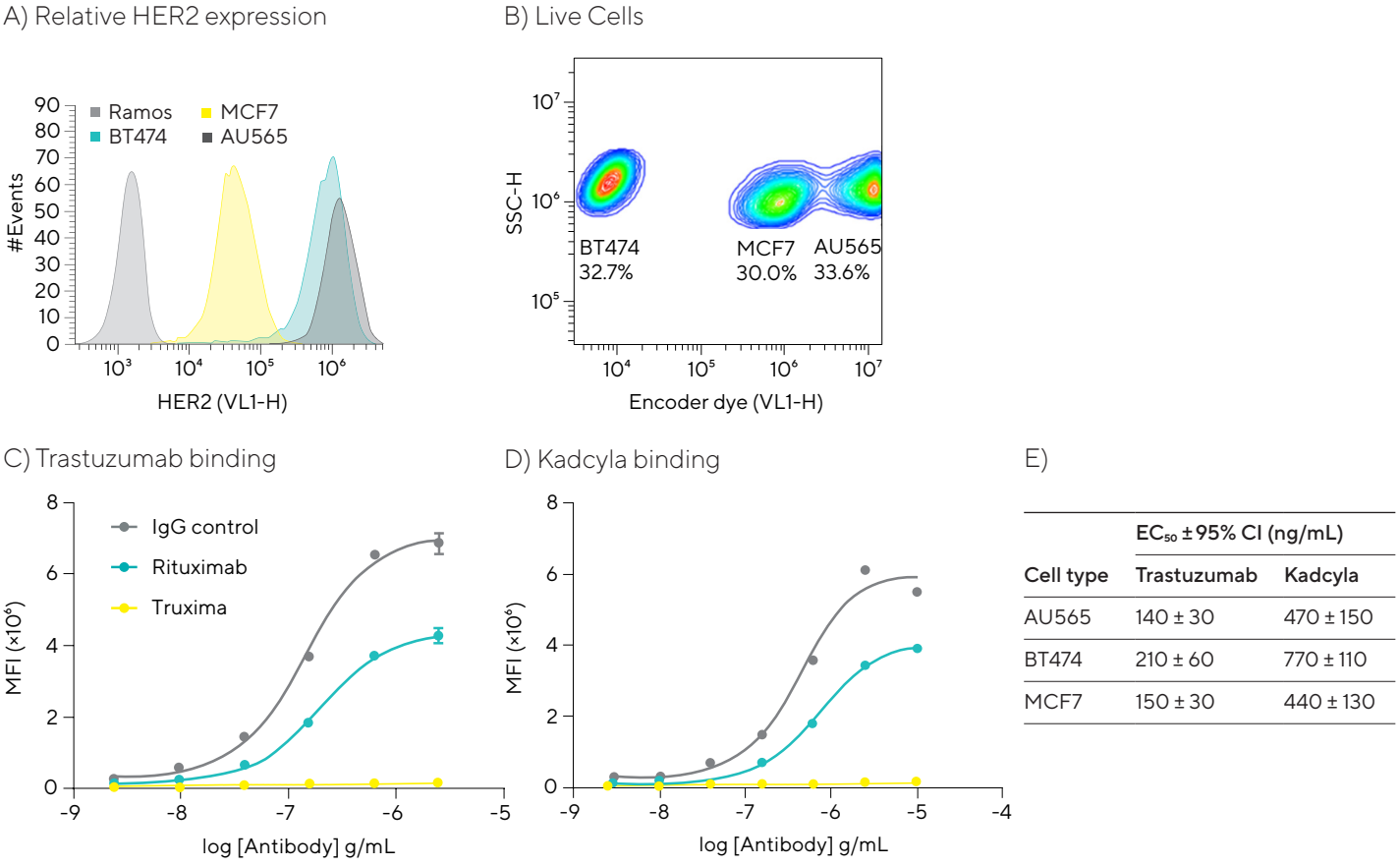
Figure 4: *Comparison of Live Cell Binding by Anti-CD20 Biosimilars*

Note: A direct mAb binding assay was used to measure the binding of anti-CD20 mAb Rituximab and one of its biosimilars, Truxima, to high CD20 expressing Raji cells. (A) Heat map of the percent of live cells that are positive for binding the antibody over a gated threshold. (B) Concentration-response curves show the % of cells positive for mAb binding over the gated threshold. (C) Table of values to describe curves in (B).

Measuring antibody binding to a range of target cells in a single well

A key factor influencing the efficacy of targeted immunotherapies, such as mAbs, for treatment of cancer, is the prevalence of the target antigen on the specific patient's cancer. To this end, expression of biomarkers such as HER2 on breast cancer cells and EGFR (epidermal growth factor receptor) on lung cancers may be tested to indicate a patient's suitability for a particular treatment[10].

It can therefore be useful to measure the binding and function of a novel therapeutic against a range of cell types, with different levels of expression of the target antigen, to give an indication of how specific patients may be affected by the treatment. To explore this using the iQue® direct antibody binding assay, we compared binding of two anti-HER2-IgG1 antibodies, a Trastuzumab biosimilar and Kadcyla (an antibody drug conjugate (ADC) based on Trastuzumab) to three adherent breast cancer cell types: AU565s, BT474s and MCF7s.



E)

	EC_{50} ± 95% CI (ng/mL)	
Cell type	Trastuzumab	Kadcyla
AU565	140 ± 30	470 ± 150
BT474	210 ± 60	770 ± 110
MCF7	150 ± 30	440 ± 130

Figure 5: *Analyze Antibody Binding to a Range of Antigen Expressing Cell Types in a Single Well*

Note: A) HER2 expression on three adherent cell lines (AU565, MCF7 and BT474) was analyzed using a conjugated anti-HER2 antibody and compared to expression on HER2-negative Ramos cells. (B) To distinguish cells in the binding assay, AU565 cells were labeled with a high concentration of iQue® Cell Proliferation and Encoder (V/Blue) Dye, MCF7 cells were labeled with a lower concentration and BT474 cells were left unlabeled. Curves plot MFI for binding of (C) a Trastuzumab biosimilar and (D) Kadcyla to the three cell lines. (D) EC_{50} values for Trastuzumab and Kadcyla binding (±95% confidence interval).

To begin with, a fluorophore conjugated HER2 antibody was used to profile the relative HER2 expression of each adherent cell type compared to Ramos cells, which were included as a HER2-negative control line (Figure 5A). The MFIs revealed highest HER2 expression on AU565 cells (MFI ($\times 10^5$) of 12.9 ± 0.08), then next highest expression on BT474 cells (MFI ($\times 10^5$) = 8.2 ± 0.2) followed by lowest expression on MCF7 cells (MFI ($\times 10^5$) = 0.4 ± 0.009). Background expression on the negative control line was negligible (MFI ($\times 10^5$) of 0.015 ± 0.003). For measurement of Trastuzumab and Kadcyla binding, like in Figure 3, the iQue® Cell Proliferation and Encoder (V/Blue) Dye was used to label cells, so that the three adherent cell types could be distinguished in a single well (Figure 5B). This facilitated simultaneous quantification of binding to each cell type.

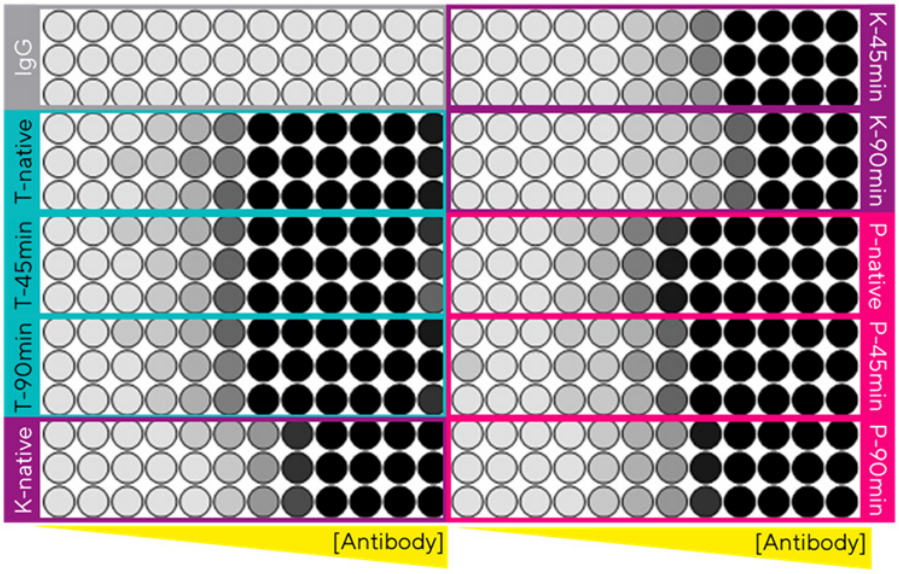
Figures 5C to E show that binding of both Trastuzumab and Kadcyla correlated with expression of HER2 on cells, with greatest binding to AU565 cells, mid-level binding to BT474s, then much lower-level binding to MCF7s.

The EC_{50} values for binding of each antibody to cells was similar, showing greater potency of binding by Trastuzumab, with EC_{50} values ranging from 140 to 210 ng/mL, compared to Kadcyla with EC_{50} values in the 440 to 770 ng/mL range.

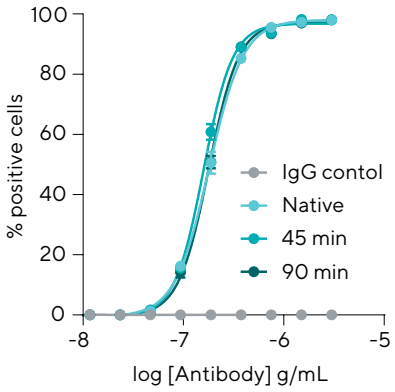
High-throughput screening of binding and thermal stability in 384-well format

High-throughput techniques are beneficial throughout the drug discovery process. They can be applied both in the early stages, where large numbers of antibodies, for example in a phage display or hybridoma library, can be screened in minimal time, and in later stages, once promising hits have been identified, to generate a full profile of their relative EC_{50} values using multiple replicates. In Figure 6, we have demonstrated the latter use of this high-throughput capability to profile binding of 10 antibodies, across 12 concentrations, with 3 replicates of each, within a single 384-well plate.

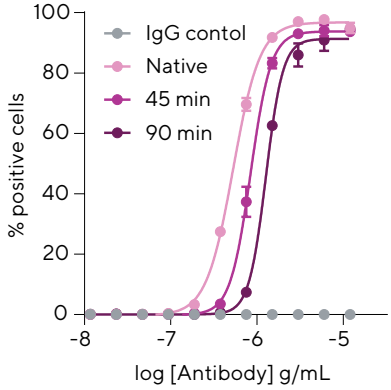
A) Secondary antibody MFI on AU565s



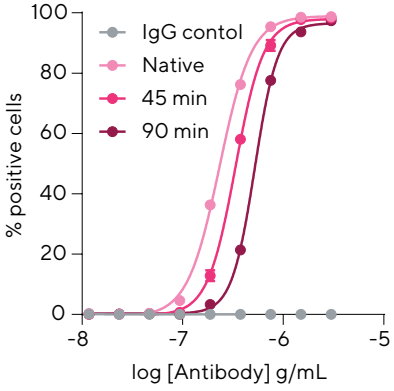
B) Trastuzumab



C) Kadcyla



D) Pertuzumab



E)

Antibody	Native	45 min heat	90 min heat
Trastuzumab biosimilar	180 ± 8	160 ± 6	180 ± 7
Kadcyla	530 ± 16	840 ± 36	1270 ± 53
Pertuzumab biosimilar	230 ± 8	340 ± 10	520 ± 12

Figure 6: *Screening Binding and Thermal Stability of Anti-HER2 Antibodies in a 384 Well Format*

Note: Unlabeled HER2-positive AU565 cells were incubated with varying concentrations of test antibodies (T= Trastuzumab biosimilar, K= Kadcyla and P= Pertuzumab biosimilar) or an IgG control (n=3). Test mAbs were tested in their native state, or after heating at 69 °C for 45 or 90 mins. (A) Heat map showing MFI for the secondary antibody on AU565 cells. Darker grey color indicates higher MFI. Concentration response curves show the % positive cells over a defined MFI threshold for the (B) native (C) 45 minute heated and (D) 90 minute heated antibodies. (E) EC_{50} values for % positive cells for binding with the native and heated antibodies.

The antibodies tested included the Trastuzumab biosimilar and Kadcyla, alongside another anti-HER2-IgG1 antibody, a Pertuzumab biosimilar. The antibodies were tested in their native state, and after 45 or 90 minutes of heating at 69 °C, with the aim of measuring their thermal stability. Binding was assessed on HER2-positive AU565 cells and compared to binding of an anti-βgal-IgG1 control.

The data in Figure 6 shows that the Trastuzumab biosimilar had both the highest level of binding and the greatest resistance to heat denaturation, with the EC_{50} remaining constant across the native, 45- and 90-minute heated antibodies, ranging between 160 and 180 ng/mL. Kadcyla displayed the lowest potency of binding to cells and was sensitive to heat denaturation, with a two-fold increase in EC_{50} from the native state (530 ng/mL) after 90 minutes of heating (1270 ng/mL). This suggests that the modification of the Fc portion to include the ADC payload, compared to the native Trastuzumab, has resulted in a loss in both thermal and cell binding stability. The Pertuzumab biosimilar had similar binding to Trastuzumab in its native state, with an EC_{50} of 230 ng/mL but it was sensitive to heating and saw a two-fold loss in binding activity after 90 minutes of heating (EC_{50} 520 ng/mL).

Competition binding assay reveals antibodies that bind to the same epitope

During antibody discovery, it is important to determine which epitope a novel drug candidate binds to on the target antigen. If it is a well characterized target for which antibodies are available with known epitope binding, this can be achieved by evaluating whether the novel antibody competes with those known binders.

This is exemplified by the data in Figure 7 which shows the results from an iQue® competition assay used to assess competition between the anti-HER2 antibodies that were profiled in Figure 6. In these experiments, a single concentration of each antibody was labeled with fluorophore conjugated secondary antibody, and the presence or absence of competition with unlabeled antibody was assessed, in a pairwise manner.

Trastuzumab binds HER2 at the C-terminal portion of domain IV, meaning Kadycla, with the same variable region should also bind domain IV[11]. Pertuzumab binds HER2 in a central region of domain II, which is thought to inhibit HER2 dimerization[11]. The data in Figure 7 support the expected epitope binding, with competition observed between Trastuzumab and Kadcyla, as indicated by the drop in MFI (left shift of the histogram relative to the IgG control) when these two antibodies were combined. The Trastuzumab biosimilar induced the greatest reduction in MFI when competing both with itself, and with the Kadcyla, which fits with the data seen in Figure 6 to suggest it is the strongest binder of the two antibodies.

There was no competition between the Trastuzumab-based antibodies and Pertuzumab as they bind different epitopes. This illustrates why these antibodies can be given as a combination therapy, which has been shown to increase median overall survival in treatment of HER2-positive breast cancers[12].

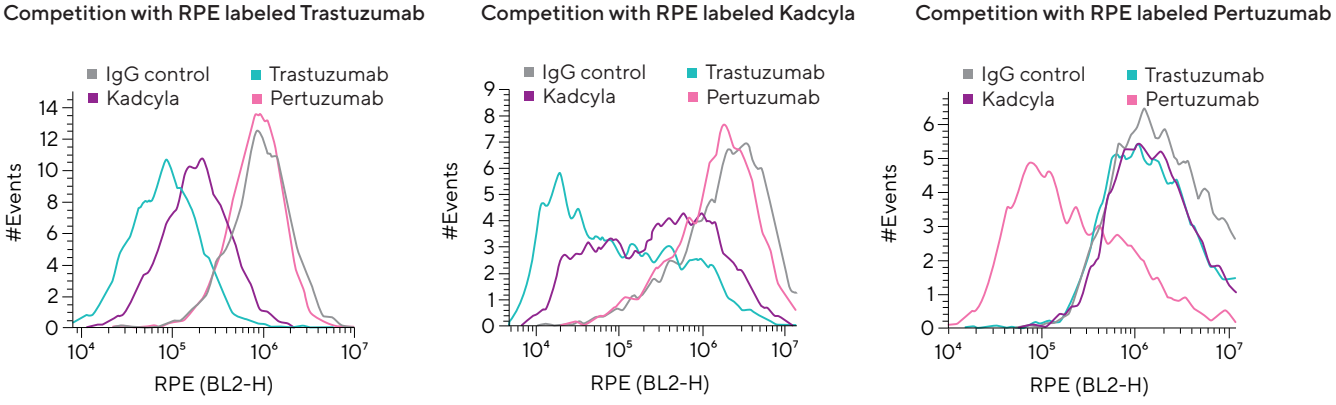


Figure 7: *Competition Binding Assay Reveals Antibodies which Bind the Same Epitope on the Target Antigen*

Note: A single concentration (1.5 µg/mL) of three mAbs (a Trastuzumab biosimilar, Kadcyla [an ADC based on Trastuzumab] and a Pertuzumab biosimilar) were labeled with RPE-secondary antibody. Labeled mAbs were paired with unlabeled mAbs (a βGal-IgG1 control, Trastuzumab, Kadcyla and Pertuzumab, 20 µg/mL) and added to HER2-positive AU565 cells. A left shift in the histogram relative to the IgG control shows a decrease in intensity of RPE, indicating competition for binding to HER2.

Conclusion

The iQue® Advanced Flow Cytometer with optimized assays enables high-throughput measurement of antibody binding activity. The direct binding workflow provides robust quantification of binding to native protein on live cells, with simple generation of pharmacological readouts, such as EC_{50} values using the iQue Forecyt® software. The competition binding assay provides a streamlined workflow to assess antibodies that bind the same epitope on the target antigen. The experiments shown in this application note have exemplified the advantages of these workflows, including:

- Easy-to-follow protocols for measuring antibody binding to target antigens on both suspension and adherent cells facilitates assessment of both solid and blood tumor targets.
- High-throughput instrumentation enables large numbers of antibodies to be profiled in minimal time and allows for enhanced replication leading to improved robustness of data. A full 96-well plate can be read in less than 15 minutes and a 384-well plate in 40 minutes.
- Low volume requirements for the iQue® allows for conservation of precious antibody samples.
- Inbuilt data analysis software streamlines data processing and speeds up the time to actionable results.
- The broad range of detection channels gives the flexibility to mix and match dyes, such as encoder or membrane integrity dyes, in a panel with your chosen secondary antibody fluorophore.

Together, these advantages create a powerful tool for measurement of antibody binding, with the potential to enhance the speed and quality of hits generated during antibody discovery.

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North America
Sartorius Corporation
300 West Morgan Road
Ann Arbor, Michigan 48108
USA
Phone +1 734 769 1600
Email: orders.US07@sartorius.com

Europe
Sartorius UK Ltd.
Longmead Business Centre
Blenheim Road
Epsom
Surrey, KT19 9QQ
United Kingdom
Phone +44 1763 227400
Email: euorders.UK03@sartorius.com

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
Email: orders.US07@sartorius.com

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Antibody discovery

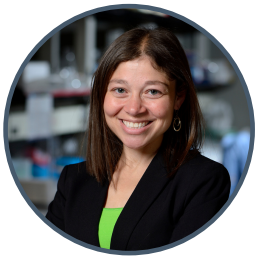
Panel Discussion: Antibody discovery

In this Panel Discussion, experts in antibody discovery discuss how the field has developed over the past 10 years, the technologies that are driving those developments and what we can expect to see in the future.

Tim Jenkins
Denmark Technical University (Copenhagen, Denmark)

Nicola Bevan
Sartorius (Royston, UK)

Lydia Fenn
LifeArc (Stevenage, UK)



Jamie Spangler
Johns Hopkins University (MD, USA)

Follow the QR code or click the link to access the discussion.

Mini webinar: Accelerating antibody discovery using advanced flow cytometry

Kirsty McBain
Sartorius (Royston, UK)

The past decade has seen an explosion in the number of antibody-based therapeutics on the market. A huge driver of this has been the advancement of technologies that improve the time and efficiency of antibody discovery. Robust, high-throughput techniques for screening antibody libraries have led to better quality hits in less time. Moreover, combining several in vitro methods to assess a broad range of antibody parameters, for example target binding and Fc function characteristics, builds a full profile of a candidate molecule and informs preclinical studies.

Follow the QR code or click the link to access the webinar.

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tfree@biotechniques.com

Business Development and Support

Commercial Director
Evelina Rubio Hakansson
e.rubiohakansson@future-science-group.com

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