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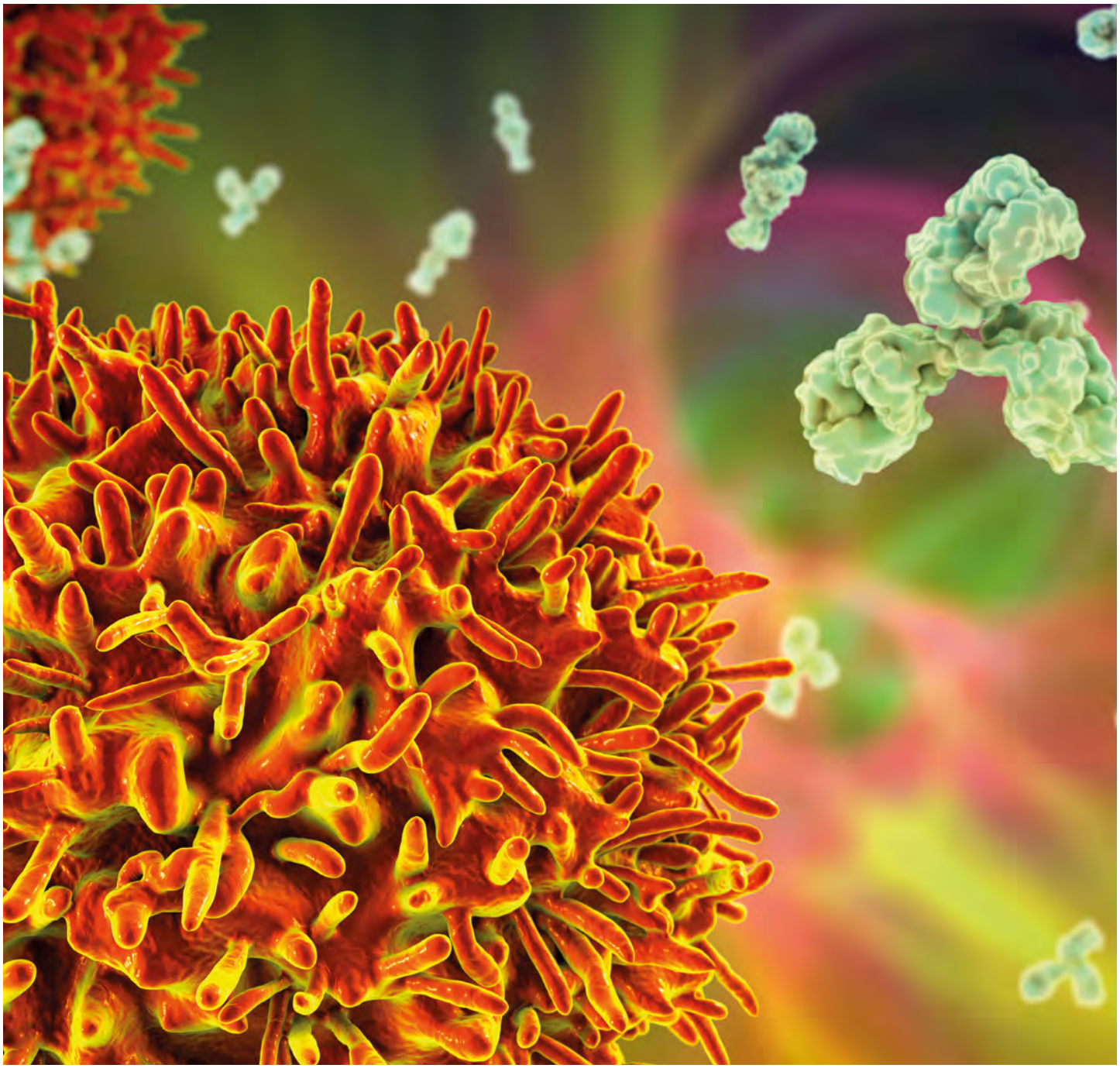

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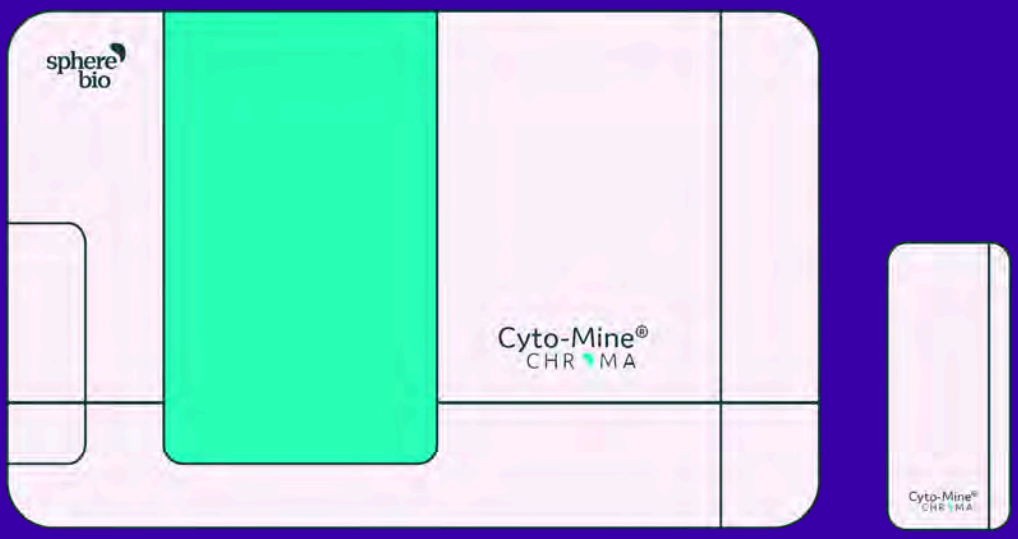

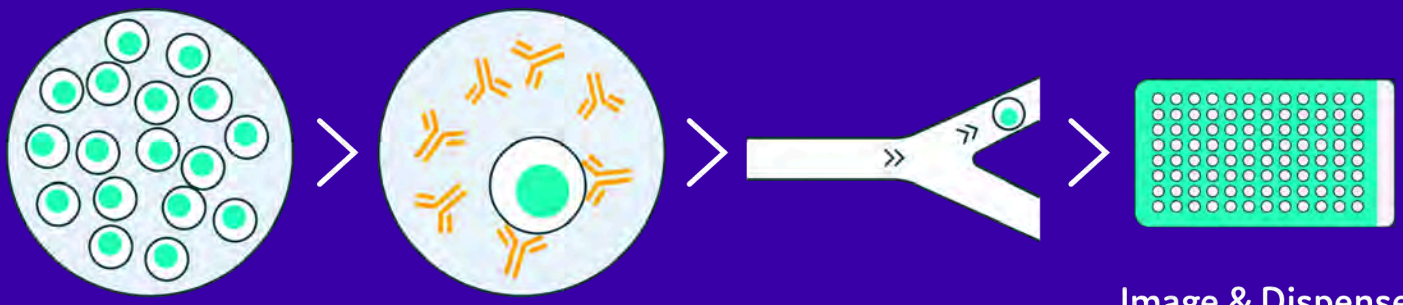

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The future for biologics discovery

By Diana Turner



Therapeutic antibodies have enjoyed huge commercial success, but where will the future take these therapies?

Precision medicines like therapeutic antibodies are expected to be a particular focus for the industry in the coming years. While these medicines have already made substantial advances in the treatment of diseases like cancer, it is likely we will see a move towards new indications. In one example, two ongoing clinical trials by Novo Nordisk are assessing IL-6 inhibiting antibody ziltivekimab for coronary artery disease (CAD).

Other industry experts predict a shift to engineered proteins coming in the year ahead. Veronica DeFelice, Director of Biologics, Sapio Sciences, explains: "In 2026, biologics discovery will increasingly focus on multispecific antibodies and engineered proteins that address complex biological targets through novel binding mechanisms. These formats are designed to interact with multiple targets or modulate disease pathways in ways that traditional monoclonal antibodies cannot."

Multiplexed single-cell analysis is one technology enabling this transition. By combining picodroplet microfluidics, multiparameter fluorescence detection and automated cell isolation, these platforms can enable scientists to examine secretion, cell identity, viability and functional capability while screening, rather than across disconnected downstream assays.

Artificial intelligence (AI), particularly generative AI is also expected to play a significant role in antibody discovery in the future. This could help overcome the main challenge in antibody development, the need to sift through a virtually infinite number of possibilities to identify the optimal sequence, and offer advances over traditional methodologies that are constrained by small-scale laboratory screens.

"2026 will mark the fusion of AI and biomaterial science," says Stella Vnook, CEO, Likarda. "AI-assisted formulation and encapsulation are transforming biologics from fragile molecules into intelligent, self-protecting systems. Adaptive hydrogel matrices now learn from real-time data to optimise release, stability, and viability across peptides, hormones, and living cells. These smart delivery ecosystems will close the gap between discovery and bedside use."

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How will the global antibody discovery market evolve in the next 10 years?

This article explores some of the factors that will shape the worldwide antibody discovery market over the coming decade.

Thanks to their low toxicity and high specificity, therapeutic antibodies have enjoyed incredible market success across a range of disease categories. The global antibody discovery market, valued at $11.15 billion in 2025, is expected to surge to $35.35 billion by 2035, growing at a CAGR of 12.23%, according to data analysts ResearchandMarkets[1]. Much like other areas of drug discovery, they predict this growth will be fuelled by advancements in engineering technologies, automation, and artificial intelligence (AI), which will further streamline antibody identification and optimisation, significantly shorten development timelines and improve cost efficiency. The specific technologies driving innovation in the antibody discovery sector will be high-throughput screening, phage display, hybridoma technology, transgenic animal models, and glycoengineering[1,2]. Another major trend shaping this growth is the shift toward fully human and humanised antibodies, which improve therapeutic safety, specificity, and efficacy while reducing immunogenicity.

Challenges and opportunities

Over the next decade, the global antibody discovery market is anticipated to progress toward the early maturity phase, characterised by broader clinical adoption, standardised workflows, and increasing regulatory clarity. As the market continues to mature, focus is shifting from innovation-driven discovery toward process optimisation, clinical validation, and cost efficiency[1]. ResearchandMarkets expects competitive dynamics to intensify as new entrants leverage computational biology and synthetic biology approaches to challenge traditional discovery models.

However, antigen identification challenges pose a significant restraint on the growth of the market[2]. The process of identifying suitable disease-specific antigens is often intricate and time-consuming. Furthermore, the absence of clearly defined antigens can hinder the development of highly specific antibodies, potentially resulting in off-target effects and diminished therapeutic effectiveness, and can also increase the risk of project failure. Overcoming these challenges requires innovative approaches, such as leveraging omics technologies and advanced screening methods, to enhance the identification and validation of relevant antigens.

> **Focus is shifting from innovation-driven discovery toward process optimisation**

Despite this, and the challenges of high production costs and competition from alternative modalities, experts believe that continued progress in automation, bioinformatics and process scalability positions the global antibody discovery market for sustained growth and technological maturity in the coming decade[1].

Regional players

ResearchandMarkets expects North America to hold the largest share of the global antibody discovery market in the decade to 2035, driven by the strong presence of major biopharmaceutical companies, advanced research infrastructure, and high R&D investments. The region benefits from early adoption of AI-driven discovery platforms, extensive clinical trial activity, and supportive regulatory frameworks that accelerate biologics development and commercialisation[1]. North America led the global market with the highest market share of 36% in 2025, figures from



Precedence Research show[2].

China is another significant player in the antibody discovery market, thanks to significant reforms in clinical development processes and regulatory reviews in the country. Precedence Research predicts Asia-Pacific will grow at the largest CAGR of 8.3% between 2026 and 2035.

In 2024, antibody-drug conjugates (ADCs) dominated oncology licensing activity in China, constituting 56% of the total deal value at $19bn, followed by monoclonal antibodies (mAbs) at 33% and small molecules at 9%, as demonstrated by GlobalData's Pharmaceutical Intelligence Center Deals Database. In one example from 2024, Merck expanded its oncology pipeline by licensing LM-299, a Phase II PD-1/VEGF bispecific antibody, from LaNova Medicines for up to $3.28bn. Globally, there are over 70 startups and small biotech companies in the ADC space, with nearly a quarter of them based in China[3]. Companies like Biocytogen Pharmaceuticals (Beijing) and Hangzhou DAC Biotech are leading the market.

The emergence of DACs

China is also at the forefront of degrader-antibody conjugates (DACs) development, which combine the specificity of antibodies with the potency of protein degraders. DACs replace the cytotoxic payload of ADCs and merge a proteolysis-targeting chimera (PROTAC) payload with a mAb via a chemical linker, aiming for targeted protein degradation and potentially improved efficacy and safety. Several Chinese companies, such as Kangpu Biopharmaceuticals, Shanghai Helioson Pharmaceutical, and Primelink Biotherapeutics (Suzhou) are prominent players in the development of DACs. According to GlobalData's Pharma Intelligence Center, there are 28 assets in development globally (Phase I: two; Pre-clinical: six, and Discovery: 20).

South Korea is another country making significant progress in the DAC sector. South Korean and Chinese companies currently have 19 assets (South Korea: 10 assets; China: nine assets) in their pipeline, according to GlobalData, with one asset in Phase I development for HER2-expressing advanced solid tumours (ORM-5029 from Orum Therapeutics, South Korea). In November 2023, Bristol Myers Squibb acquired potential first-in-class DAC ORM-6151 (currently in Phase I) from Orum for approximately $180m.

In July 2024, Vertex Pharmaceuticals and Orum entered into a global collaboration agreement to develop novel DACs.

In the US, many big players have already joined the race to develop DACs. In 2023, C4 Therapeutics entered a collaboration agreement with Merck for DACs, where C4 will get $10m upfront, milestones that could total $600m and about $2.5bn across the collaboration. Also in 2023, Seagen (now part of Pfizer) and Nurix Therapeutics entered a collaboration worth more than $3.4bn for DAC development.

The future of antibody discovery

Complex, multi-specific antibodies and ADCs are expected to dominate the market going forward thanks to their application in treating cancers, but also potential new opportunities in autoimmune diseases and infectious diseases. However, the expansion of ADCs is partially limited by the high cost associated with their research and development, which excludes small-scale pharma and biotech companies from taking a share. Low penetration capacity can also be an issue, alongside immunogenicity, lack of stable linkage in blood circulation, drug resistance, uncertain toxicity, poor mechanism of penetration and unusual size of mAbs. However, it is hoped that next-generation ADCs like DACs, antibody-oligo conjugates (AOCs) and radioconjugates (RDCs) can combat such limitations by improving overall efficacy.

Biswajit Podder, Oncology and Hematology Analyst at GlobalData, comments: "ADCs face several challenges, including resistance to the payload, narrow therapeutic window, molecular heterogeneity, and toxicity, such as interstitial lung disease and thrombocytopenia. However, by identifying novel targets, developing bispecific antibodies, engineering better linkers, using multiple different payloads on one ADC, and identifying suitable patient populations using biomarkers, ADCs have the potential to replace chemotherapy, improve safety and efficacy, and prevent resistance mechanisms by combining ADCs with other treatment modalities such as immunotherapy."

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What cells do, not what they look like: Rethinking B cell screening for antibody discovery

How are new technologies improving efficiency and downstream success rates in antibody discovery?

Antibody discovery sits at the foundation of modern therapeutics. From oncology and autoimmune disease to infectious disease and immunomodulation, monoclonal antibodies underpin some of the most effective and widely used treatments in medicine. Yet despite their clinical impact – and the technological advances that now enable rapid sequence generation and analysis – the process of identifying the right antibody remains inherently constrained[1–3].

Over the past decade, upstream innovations in immunisation strategies, sequencing, and computational biology have transformed how researchers access and characterise immune repertoires. These advances have significantly expanded the antibody repertoire diversity available to discovery teams around the world. What they have not solved, however, is the central challenge that defines success: identifying, within that diversity, the rare cells that produce clinically relevant antibodies[4].

At its core, antibody discovery is a problem of both scale and resolution. Theoretically, the human B cell repertoire can generate between 10^{12} - 10^{18} different B cell clones. In reality, the number of different B cell clones within an individual is around 1 x10^7 – 2 x10^7, each with distinct receptor sequences and biological potential[5]. Within this population, only a small subset produces antibodies with the affinity, specificity, and molecular properties required for therapeutic development[4]. Increasingly, these candidates must also demonstrate functional relevance – such as target cell receptor blockade, pathway modulation, or immune activation – attributes that extend beyond simple binding and are not captured by conventional screening approaches[6].

Why traditional screening struggles

For decades, antibody discovery has relied on a combination of approaches such as fluorescence-activated cell sorting (FACS), hybridoma generation, and limiting dilution cloning. These methods have delivered many successful therapeutics and remain widely used. However, they were not designed to interrogate antibody secretion directly at single-cell resolution[2].

Instead, they rely on phenotypic characteristics – surface markers, binding signals, or growth behaviour – to identify candidate cells. While useful for enriching populations, these readouts provide only indirect insight into functional performance[2]. These methods can determine whether a cell belongs to a specific lineage or binds a target under certain conditions, but they do not resolve how an antibody behaves in a biological context.

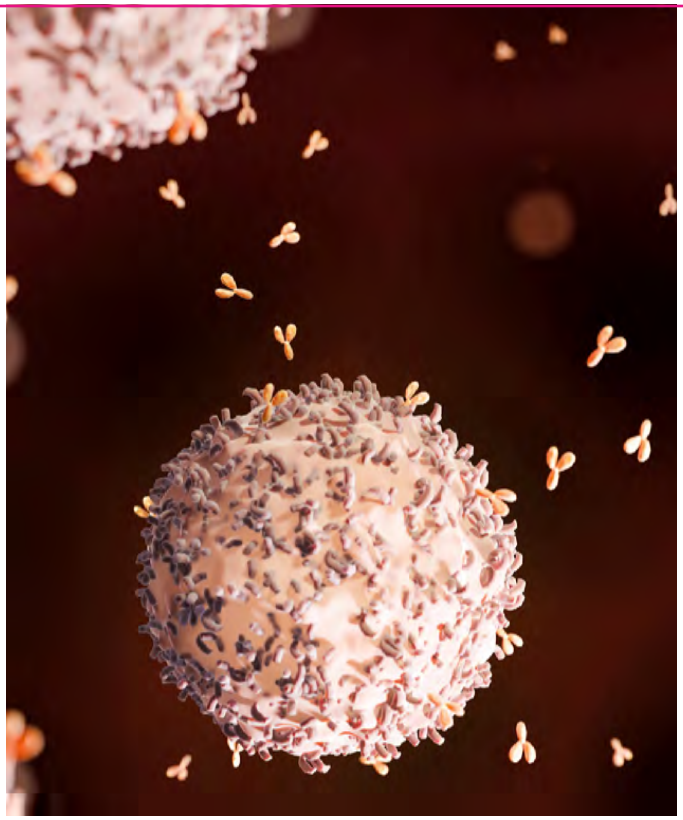
This distinction is becoming increasingly important. In many therapeutic areas, particularly in oncology and immune modulation, binding alone is insufficient. Antibodies must exert defined biological effects – blocking receptors, triggering signaling cascades, or modulating cellular interactions – none of which can be inferred reliably from phenotype characterisation alone[6]. Therefore, surface phenotype offers, at best, an incomplete proxy for its multifaceted roles, introducing uncertainty at the earliest stage of selection.

Secretion as a missing measurement

In conventional workflows, antibody secretion – the most direct measure of B cell output – is not assessed during selection. Instead, it is evaluated later through downstream assays such as ELISA or functional bioassays[7].

This characterisation process between selection and validation creates both practical and conceptual challenges. When using hybridoma technology, cells are sorted, expanded, and often pooled before their antibodies are characterised, weakening the connection between the originating cell and its functional output. During this process, rare or slow-growing clones may be lost, and additional variability might be introduced through culture and handling.

As discovery programmes increasingly prioritise functional activity – such as pathway inhibition or immune engagement – this delay becomes more limiting[6]. Critical information is only obtained



after key decisions have already been made, reducing efficiency and increasing the likelihood of missed opportunities.

Rare cells and cumulative loss

The limitations of indirect screening are compounded by the inherent rarity of high-value cells. Within large B cell populations, the fraction of cells producing therapeutically relevant antibodies may be exceedingly small[4]. When additional criteria such as mechanism of action or functional efficacy are applied, that fraction narrows further[6].

Traditional workflows introduce loss at multiple stages. Hybridoma generation can be inefficient and biased and limiting dilution is time-consuming making the workflow difficult to scale[2]. Each step reduces the number of cells carried forward, and with it, the diversity available for evaluation.

By the time functional screening occurs, the original repertoire has often been substantially reduced. In practical terms, this means that potentially valuable antibodies may never be observed.

Function-first screening

Emerging technologies are addressing these constraints by reframing the problem: shifting from phenotype-based selection to direct measurement of cellular function.

Rather than inferring performance from surface characteristics, these approaches focus on the ability of individual cells to produce antibodies with defined biological activity. Central to this shift is the capability to measure secretion at the single-cell level, enabling the capture of both quantitative output and functional properties within the same workflow[6,8].

Droplet-based microfluidic systems have become a key enabler of this approach. By isolating individual cells in picoliter-scale compartments, they create controlled environments in which secreted antibodies can be accumulated, detected, evaluated, and isolated in real time[8,9]. This allows functional screening to occur at the point of origin, preserving the link between each cell and its antibody.

Droplet-based microfluidics: Enabling single-cell functional assays

Each droplet in a microfluidic system acts as an independent microreactor, containing a single B cell and the necessary reagents for detection and analysis[9]. Within these confined volumes, secreted antibodies rapidly reach detectable concentrations, enabling sensitive measurement without the dilution effects seen in bulk assays.

This localised environment supports not only detection of secretion but also interrogation of function. Binding activity, inhibition, or other biologically relevant effects can be assessed within the droplet, providing a more complete picture of antibody behaviour[9,10].

Importantly, the association between cell and output remains intact. Each functional signal can be traced directly back to the originating cell, allowing precise identification, isolation, and downstream analysis of candidates with defined properties[9].

A defining advantage of droplet microfluidics is its ability to scale. Modern systems can generate, analyse, and sort millions of droplets within a single experiment, enabling comprehensive interrogation of B cell repertoires at single-cell resolution[9].

This combination of throughput and functional insight fundamentally changes how discovery can be performed. Large populations can be screened without sacrificing biological relevance, and selection criteria can extend beyond simple binding to incorporate multiple functional parameters[6].

As a result, researchers are no longer limited to identifying antibodies that interact with a target – they can prioritise candidates based on how they behave, improving both efficiency and downstream success rates.

Conclusion

The next phase of antibody discovery will be defined by the ability to connect biological function directly to individual cells at scale. Approaches that enable this connection are already reshaping how candidates are identified and prioritised.

By bringing functional measurement upstream – linking secretion, activity, and cell identity within a single workflow – these technologies reduce uncertainty, accelerate timelines, and improve the probability of selecting clinically relevant antibodies[6].

The central question is no longer what a cell looks like, but what it does.

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Find rare antibodies faster with integrated single-cell screening power

Antibody discovery doesn't fail because of a lack of candidates. It fails because the right ones aren't recognised early enough. Cyto-Mine® and Cyto-Mine® Chroma bring secretion into the first screening decision, enabling high throughput, single cell analysis and earlier selection of antibody candidates with clearer downstream potential.

The screening challenge

Antibody discovery workflows have historically relied on indirect signals. Fluorescence activated sorting, hybridoma fusion and limiting dilution can enrich populations, but they typically prioritise surface phenotype or growth behaviour over what a cell actually secretes. When secretors are extremely rare, proxy based selection can under-sample the long tail and allow high value candidates to disappear into the background.

A second consequence of proxy first workflows is delay. Cells are sorted, expanded and only then tested in downstream assays. Each handoff adds time, variability and attrition – especially when working with precious primary material.

Bringing function forward with picodroplets

Cyto-Mine® is designed to bring functional information into the earliest screening decision.

The platform uses picodroplet microfluidics to compartmentalise individual cells into tiny, cell friendly reaction vessels. Within each picodroplet, secreted antibody can be detected using fluorescence assays – so each cell is assessed by function (secretion) rather than phenotype alone. In a single automated run, Cyto-Mine® integrates encapsulation, incubation, assay, imaging, sorting and dispensing of viable cells, maintaining the link between the secreting cell and its assay readout for downstream recovery.



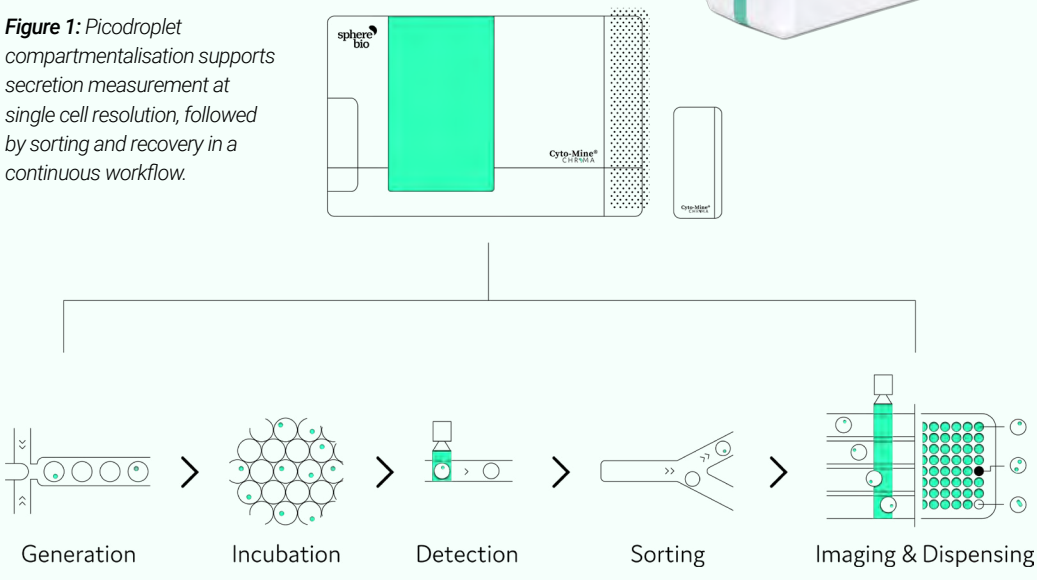

Figure 1: *Picodroplet compartmentalisation supports secretion measurement at single cell resolution, followed by sorting and recovery in a continuous workflow.*



Figure 2: *Cyto Mine® Chroma adds four colour detection to enable multiplexed single cell screening and earlier candidate selection decisions.*

Key takeaways (for B cell screening)

- Move function earlier: measure secretion at single cell resolution during screening, not after expansion.
- Screen at scale: analyse millions to tens of millions of cells to improve the chance of capturing rare secretors.
- Reduce cycle time: published programmes report four weeks from cells to confirmed recombinant antibodies.
- Add multiplexing: four colour detection supports multi parameter selection in a single run.

By miniaturising assay volume and automating handling, the approach supports screening at population scales that are difficult to reach with conventional methods. Cyto-Mine® is routinely used to functionally analyse millions of cells, and pooled workflows can extend that to tens of millions in a day – helping teams sample deeper into the population where the 'one in a million' secretors reside.

A timeline that supports decisions

Earlier functional screening is only meaningful if it shortens the path to confirmed antibodies.

Multiplexing with Cyto-Mine® Chroma

As targets become more complex, a simple "binds" gate is rarely sufficient. Researchers increasingly need to understand multiple attributes without adding extra cycles of expansion and re testing.

Cyto-Mine® Chroma extends Cyto-Mine® capabilities to allow four colour detection, enabling multiplexed readouts within a single run. This allows screens that answer more than one question at the point of selection – for example selecting for preferred isotype or subclass, or carrying forward only viable, productive secretors.

The practical benefit is decision quality. When multiple parameters are measured together, it becomes easier to prioritise candidates with fewer late stage surprises and reduce the downstream burden of additional screening.

Maintaining traceability from droplet to well

High throughput functional screening must still support recovery and traceability.

Cyto-Mine® workflows are designed to preserve viability during processing and to support documented evidence of monoclonality through imaging at dispense. Recovered cells can be taken directly into sequencing and expression workflows.

In independent published work using a Cyto-Mine®–enabled workflow, Biointron[3] researchers reported ~90% sequence recovery efficiency and 100% reconfirmation of selected candidates, indicating that very high throughput screening can remain transparent and reproducible when moving from single cells to expressed antibodies.

Case example: Merck KGaA: functional screening to confirmed antibodies in four weeks

Researchers screened millions of primary B cells and progressed from cell preparation to confirmed recombinant antibodies in four weeks, yielding 44 sequence diverse, high affinity antibodies in a single campaign[1,2]. The same work reported substantial enrichment of functional cells in a single Cyto Mine® run turning low starting hit rates into highly enriched populations ready for recovery, sequencing and expression.

For teams managing multiple discovery programmes, this kind of compression matters operationally. It reduces the number of iterative rounds required to converge on a set of candidates and brings 'go/no go' information forward to a point where it can still shape experimental strategy.

Summary

For antibody discovery from primary B cells, the most valuable information is what each cell secretes. Cyto-Mine® and Cyto-Mine® Chroma enable functional, single cell screening at scale, combine selection and recovery in one workflow, and add multiplexed readouts to support earlier, better informed decisions.

See how integrated screening can transform your discovery process.

- Speak with a specialist
- Explore application workflows
- Learn more about Cyto-Mine® Chroma

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Rapid and efficient screening of primary B cells to identify target-specific antibodies

The advancement of therapeutic antibody discovery faces time and cost challenges. Merck R&D scientists address this by employing Sphere Bio's microfluidics picodroplet technology. Rapid high-throughput screening of primary antibody-secreting cells from rodent and human donors, demonstrating specific antibody binding to recombinant antigens, showcases the streamlined and automated workflow of Sphere Bio's Cyto-Mine platform in antibody discovery projects.

The first therapeutic monoclonal antibody (mAb), muromonab-CD3, was approved for clinical use by the US Food and Drug Administration (FDA) in 1986[1]. Since then, over 190 therapeutic antibody drugs have been approved worldwide, and hundreds more are currently under development[2].

Conventional mAb production methods such as hybridoma technology and display techniques are low-throughput, time-consuming and resource-intensive[3]. Modern display techniques offer greater immune diversity, however, require the need for tedious subcloning of surface-displayed hits into soluble formats, leading to loss of diversity in the final library[4]. Both these methods do not require the structure of a target to be known for antibody development, leading to downstream uncertainty in terms of safety[5]. Compared to these limitations, droplet-based microfluidics technology offers the unique advantage of encapsulating individual antibody secreting cells (ASCs) in miniaturised water-in-oil droplets with unprecedented throughput. Cell encapsulation in droplets provides a protective environment offering high cell viability and assay sensitivity across a wide range of cell types, including primary B cells from animals and human donors.

Moreover, antibodies secreted by droplet-encapsulated ASCs retain their genotype-phenotype linkage and their native chain pairing, which can be analysed by next-generation sequencing and used for direct antibody subcloning or subsequent library generation. As such, droplet-based microfluidic technologies, such as the Cyto-Mine®, are opening up new avenues for the discovery of antibodies with unique functional properties or against difficult targets, i.e., insoluble membrane proteins[6-8].

Leveraging picodroplet-based technology, Cyto-Mine can be used to efficiently isolate and analyse up to 40 million cells with antigen specificity by encapsulating them into individual picodroplets, assaying for the target antigen, selecting for rare antigen-specific ASCs,

Table 1. Composition of the picodroplet encapsulation medium

Encapsulation medium component	% (v/v)
Cell culture medium	83%
OptiPrep™ Density Gradient Medium	16%
Pluronic™ F-68 (10%)	1%

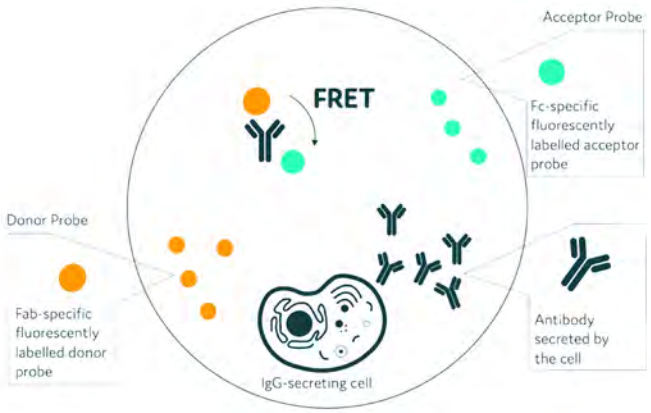

Figure 1: *Positive events readout using the Cyto-Mine picodroplet-based FRET IgG secretion assay. This model shows the standard assay to screen for IgG secreting cells. A customised pair of IgG-specific fluorescent probes are trapped within each picodroplet during encapsulation. IgG secreted from the encapsulated cell is recognised by the detection probe pair forming a 3-body FRET complex that induces a shift in fluorescent signal.*

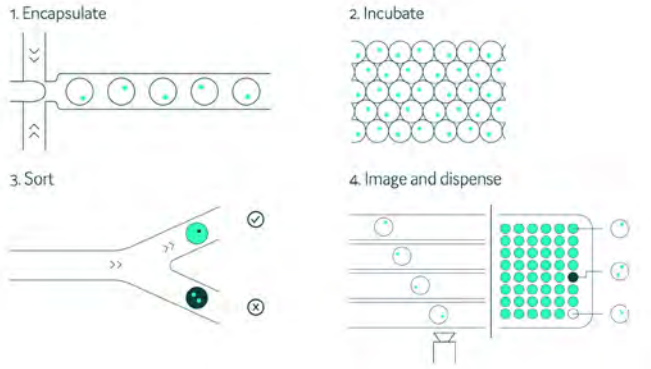

Figure 2: *The Cyto-Mine workflow integrates the screening, sorting, isolation and verification of antigen-specific clones into a fully automated process.*

and dispensing as single cells into 96- or 384-well microtiter plates (MTPs) for downstream functional analysis in just one day.

This app note demonstrates how Cyto-Mine was used by Merck's R&D scientists for the rapid identification of target-specific antibodies from B cells isolated from rodents or human donors screened for antibody secretion, specific binding to recombinant antigens or antigen-positive cells, and directly for functional studies[9].

Materials and methods

Plasma cell preparation:

Human plasma blasts were isolated from the peripheral blood of healthy donors six or seven days post tetanus toxoid (TT) vaccination. Isolation of peripheral blood mononuclear cells (PBMCs) was performed by following the SepMate™-50 IVD (StemCell) manual. Pan-B enrichment of PBMCs was carried out using the EasySep™ human Pan-B cell enrichment kit (StemCell) and a RoboSep device (StemCell). Then, CD38$^+$ cells were separated using a CD38 monoclonal antibody (HIT2), Biotin, eBioscience™ (Invitrogen), in a concentration of 0.5µg/100µl in combination with EasySep human biotin positive selection kit II (StemCell).

Primary mouse plasma cells, from bone marrow, lymph nodes and splenocytes, were prepared five-days post boost with a recombinant C-terminal domain tetanus toxoid fragment (TT-CTD) or an extracellular domain of a Cancer Related Antigen (CRA). Cells were enriched for ASCs by EasySep Mouse CD138 Positive Selection Kit (StemCell) using a RoboSep device (StemCell). Finally, isolated human CD38$^+$ or murine CD138$^+$ cells were transferred to fresh cell medium before preparation for Cyto-Mine analysis.

Plasma cell screening

To sort for plasma cells secreting antigen-specific antibodies, one million ACSs were resuspended in 1mL of the encapsulation medium, as shown in Table 1. In FRET-based experiments, used to screen for antibody secretion and recombinant antigen binding, AlexaFluor488 labelled TT-CTD and Goat F(ab')2 Anti-Human IgG-(Fab')2 (DyLight-594) as the FRET donor and acceptor, respectively, were added to reach 1mL of the basic encapsulation medium for screening human CD38$^+$ cells secreting antibodies against TT (Fig 1). To sort CD138$^+$ murine plasma cells secreting antibodies against biotinylated CRA, cells were resuspended in 1mL of encapsulation medium with Streptavidin-AF488 labelled with biotinylated CRA and Goat F(ab')2 Anti-Mouse IgG – (Fab')2 (DyLight® 594) as FRET donor and acceptor, respectively.

Cyto-Mine workflow for sorting ASCs for antigen-specificity

The Cyto-Mine workflow integrates single-cell encapsulation, incubation, screening, sorting, isolation, and verification of target-specific ASCs in a fully automated process (Fig 2). The cell suspension of one million ASCs was filtered through a 40µM cell strainer before being pipetted into a Cyto-Cartridge® and loaded into the Cyto-Mine to initiate cell encapsulation. Cells in 1mL were encapsulated in two million 450pL droplets that were immediately incubated *in situ* at 37°C to allow for secretion of antibodies within the picodroplets to reach a detectable level for

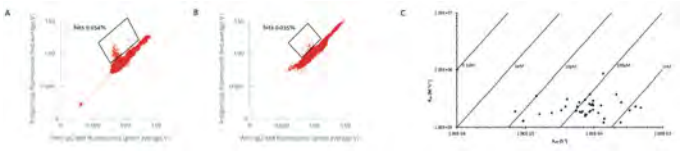

Figure 3: *Sorting of freshly sourced and revived human ASCs for TT target specificity (adapted from Gaa et al ., 2021, under CC BY 4.0 License). Sorting Scatter plot obtained from, A) human ASCs, and B) revived human plasmablasts secreting antibodies against TT via FRET: a decrease in green and an increase in red fluorescence indicates FRET by recombinant target binding in the respective droplets. C) TT binding affinities via BLI showing mainly sub-nanomolar TT affinities of binders obtained via Cyto-Mine within four weeks from ASC sourcing.*

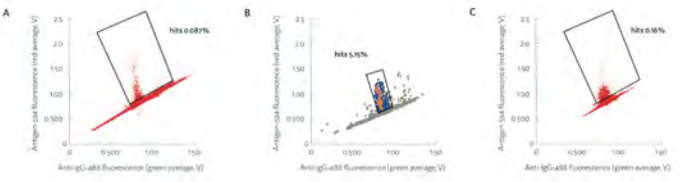

Figure 4: *Sorting of fresh and revived murine ASCs for Antigen-594 fluorescence (red average; V) 2.5 CRA target specificity (adapted from Gaa et al ., 2021, under CC BY 4.0 License). A) Sort of freshly obtained murine plasma cells for secreted antibodies specific for a CRA. B) Dispensing of sort shown in (A) – binders later confirmed as positive are shown in red, dispensed droplets in light blue, and non-dispensed droplets in gray that were used as a negative reference signal during dispensing. Positions of positive signals confirm appropriate gating. C) Sort for CRA specificity as in (A), applying frozen and revived plasma cells. The statistics of these antibody hit discovery campaigns are shown in Table 2.*

Antigen	TT		CRA	
ASC Source	Freshly prepared	5 days Frozen/thaw	Freshly prepared	5 days Frozen/thaw
MACS output	5,230,000		6,520,000	
Viability (%)	98	99	95	92
Percentage ASCs/ Total cells (%)	n.d.	n.d.	11	9
Number of droplets/cells	$2*10^5/2*10^5$	$1*10^5/1*10^5$	$2*10^5/1*10^5$	$2*10^5/1*10^5$
Number of cells sorted	823	221	~2,300	~3,000
Number of cells dispensed	316	117	635	96
Full mAb gene PCR amplicons yielded	83	47	98	33
Number of mAbs produced	72		92	n.d.
Number of target-specific mAbs	44		30	n.d.

Table 2: *Screening of human and mouse ASCs for target specificity by Cyto-Mine (adapted from Gaa et al ., 2021, under CC BY 4.0 License). Statistics for two Cyto-Mine antibody discovery campaigns. Magnetic-activated cell sorting (MACS) output in the TT campaign represents CD38⁺ enriched plasma blasts obtained from 297mL of one healthy vaccinated donor's peripheral blood, whereas CRA campaign MACS output originates from pooled bone marrow, lymph node, and spleen samples of two immunised wild-type mice enriched for CD138+ plasma cells. Of 1050 and 1354 CRA-specific droplets dispensed, 635 and 96 were analysed and yielded 98 and 33 full monoclonal antibody (mAb) gene PCR amplicons, respectively. 33 of 2-month campaign were not further processed. The difference between the number of sorted and dispensed cells arises from the amalgamation of positive and negative droplets during sorting to fulfil a droplet requirement. Despite implementing gating strategies for consistency, divergences in photomultiplier tube settings introduce variations between the sorting and dispensing modes.*

the FRET signal to develop. The miniaturised format enables an ultrasensitive, rapid assay of typically 0.5 to two hours depending on the production rate of the cell population.

Cyto-Mine measured the fluorescence of each picodroplet and, in a similar manner to flow cytometry, antigen-positive hits were sorted for collection and stored in a chilled microchamber prior to dispensing. Just before dispensing, the Cyto-Mine imaged each droplet five consecutive times providing visual evidence of a single-cell progenitor into individual wells of 96- or 384-well MTPs prefilled with the lysis buffer.

Results

Screening for human ASCs

As an example of Cyto-Mine's high throughput and ability for human diversity mining of plasma blasts, three million ASCs purified from peripheral blood of TT-vaccinated human donors were screened for TT-antibody specific secretion either on the same day of sourcing or frozen for five days before screening with Cyto-Mine (Figs 3A and B, respectively). One thousand droplets were sorted for antigen-specificity and 433 droplets were dispensed as single cells into final MTPs, and subjected to single-cell PCR analysis to retrieve IgG sequences, and subsequent validation for specific binding was confirmed using biolayer interferometry (BLI) (Fig 3C).

The analysis revealed that the Cyto-Mine-based antibody discovery campaign yielded a panel of 44 sequence diverse and high affinity fully human TT antibodies from plasma blasts enriched from the peripheral blood of a TT vaccinated human donor in less than one month (Table 2).

Screening of rodent ASCs

To demonstrate the applicability of a similar streamlined workflow for assessing immune diversities in rodents, ASCs isolated from rodents pre-immunised with a CRA underwent the same screening process. The use of Cyto-Mine's picodroplet technology facilitated the encapsulation of CD138⁺ plasma cells in droplets, which were then screened at the single-cell level for antigen specificity (Fig 4). Of the droplets screened, 2,300 were found to contain antigen-specific ASCs. From these, 635 were chosen for further downstream analysis. This process resulted in 98 full monoclonal antibody gene PCR amplicons, which were then subcloned and produced. Out of these, thirty were confirmed to be target-specific (Table 2).

Conclusions

In conclusion, these data show the power of Cyto-Mine for efficiently screening millions of primary B cells, enabling Merck R&D scientists to identify highly specific TT and CRA antibodies. The complete antibody discovery process was completed in just 28 days, with Cyto-Mine being used on day one for the rapid screening, isolation and single-cell analysis of antigen-specific B cells, followed by 27 days for gene synthesis, single-cell cloning, production, and validation.

Compared to traditional B cell mining methods, Cyto-Mine enables high-throughput screening within the same day of cell isolation. Antigen-specific B cells are commonly isolated via flow cytometry[10]. However, flow cytometry can be very harsh on cells leading to significant cell loss, and limited to membrane-bound antibodies. Water-in-oil picodroplets provide a uniquely protective environment to support cell viability and integrity since the aqueous phase is made up of specific cells grown medium, and the proprietary formulations of Cyto-Mine surfactants protect them from shear stress as they flow through the microfluidic channels. Thus, Cyto-Mine's picodroplet-based process is gentle on the selected cells.

In addition to the single well export of the Cyto-Mine screening workflow shown here, Cyto-Mine's picodroplet-based technology allows dispensing in bulk for possible re-compartmentalisation for NGS analysis of IgG repertoire analysis, facilitating the interrogation of both a spatial and temporal immune response.

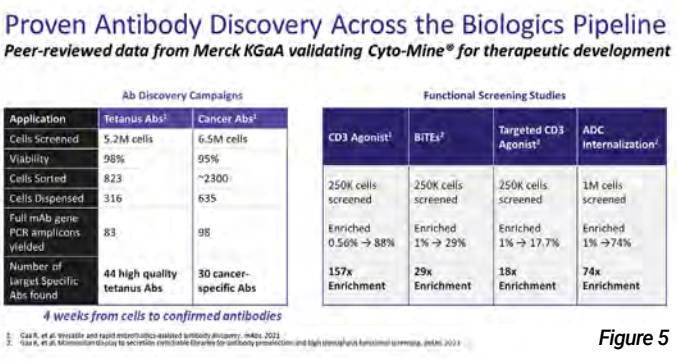

Figure 5

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Multiplexed rapid B cell screening and the future of antibody discovery

Antibody discovery is no longer constrained by our ability to generate large numbers of candidates – the limiting factor has shifted to recognising the best candidates early enough to shape downstream decisions.

As therapeutic programmes become more complex, the central question has shifted from 'How many cells can we screen?' to 'How much can we understand about each cell, and how quickly can we act on that insight?'

Multiplexed single-cell analysis is changing the answer. By combining picodroplet microfluidics, multiparameter fluorescence detection and automated cell isolation, platforms such as Cyto-Mine® Chroma enable scientists to examine secretion, cell identity, viability and functional capability while screening, rather than across disconnected downstream assays. The result is not simply faster screening but enabling easier and richer characterisation of rare cells before valuable biology is lost.

Revisiting the foundations: From hybridomas to single B cells

The modern antibody discovery field was built on the success of hybridoma technology. For decades, hybridomas provided a reliable route to monoclonal antibodies, preserving native heavy and light chain pairing and enabling reproducible antibody production.

> **Discovery becomes a continuous, data-driven flow rather than a fragmented pipeline**

Yet hybridoma workflows remain inherently constrained:

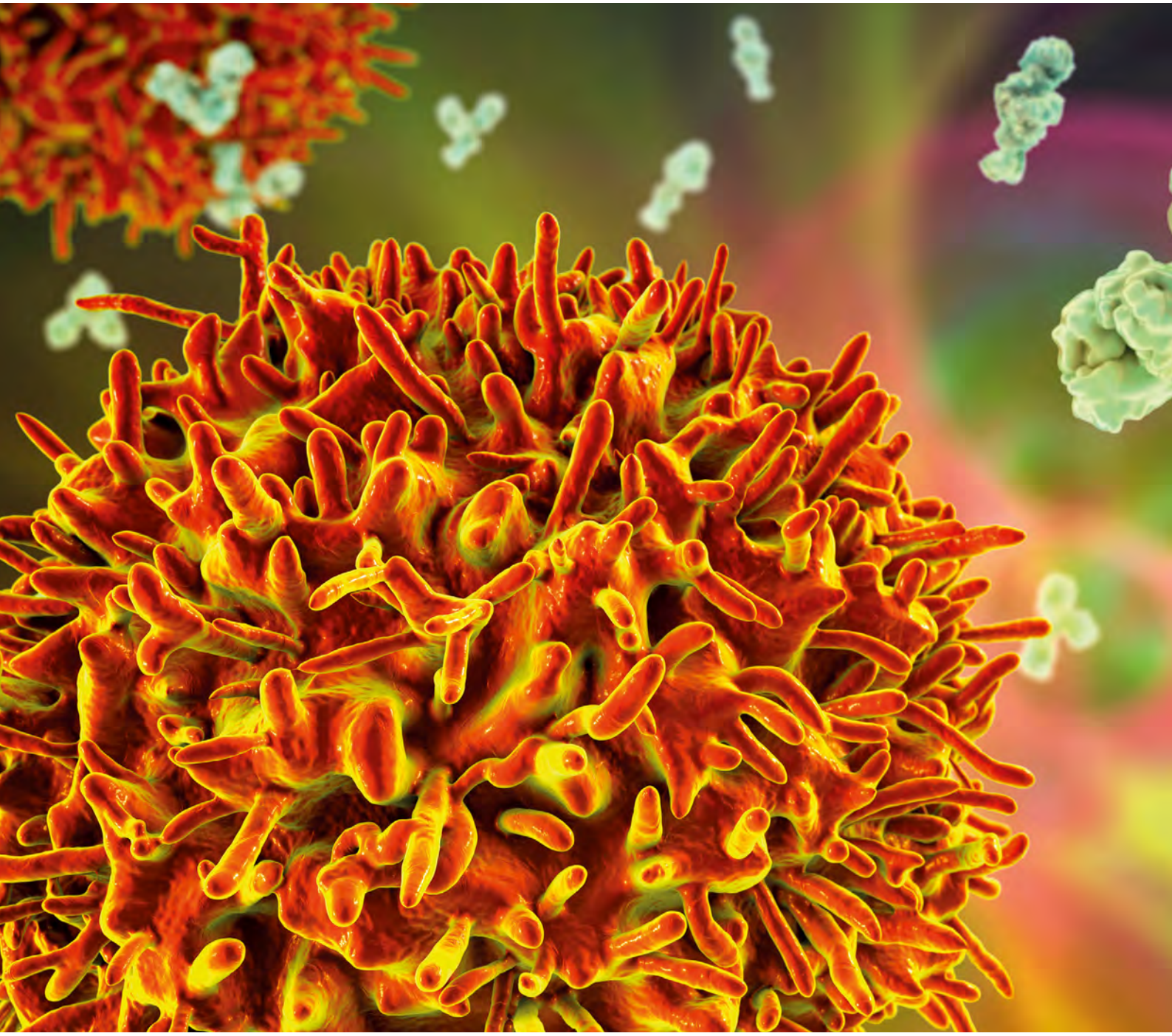
- Restricted screening throughput
- Extended timelines
- Loss of rare high-value B cells

The latest inflection point is the move from single B cell screening as a throughput exercise to single cell screening as a characterisation workflow. Multiplex platforms such as Cyto-Mine Chroma integrate encapsulation, assay readout, sorting, imaging and dispensing into a connected process, allowing scientists to characterise cells while maintaining the link between the single cell and its antibody output.

Beyond throughput: The shift to multi-dimensional screening

Ultra-high throughput single cell screening helps accelerate antibody discovery, but antibody quality is rarely defined by one measurement. A strong candidate may need to combine secretion, antigen specificity, cell viability and functional activity. Screening millions of cells is only valuable if the workflow can distinguish the rare cells that satisfy multiple criteria at once.

Cyto-Mine Chroma strengthens this decision point through four-laser, four-channel fluorescence detection. This expanded optical capability enables researchers to capture multiple assay signals within picodroplets, supporting workflows that can differentiate antibody secreting cells, target



cells, secretion, antigen binding etc. in a single experiment.

This changes the purpose of early screening: instead of creating a broad hit list, build a multi-dimensional profile of each candidate.

Multiplexing: Seeing more, earlier

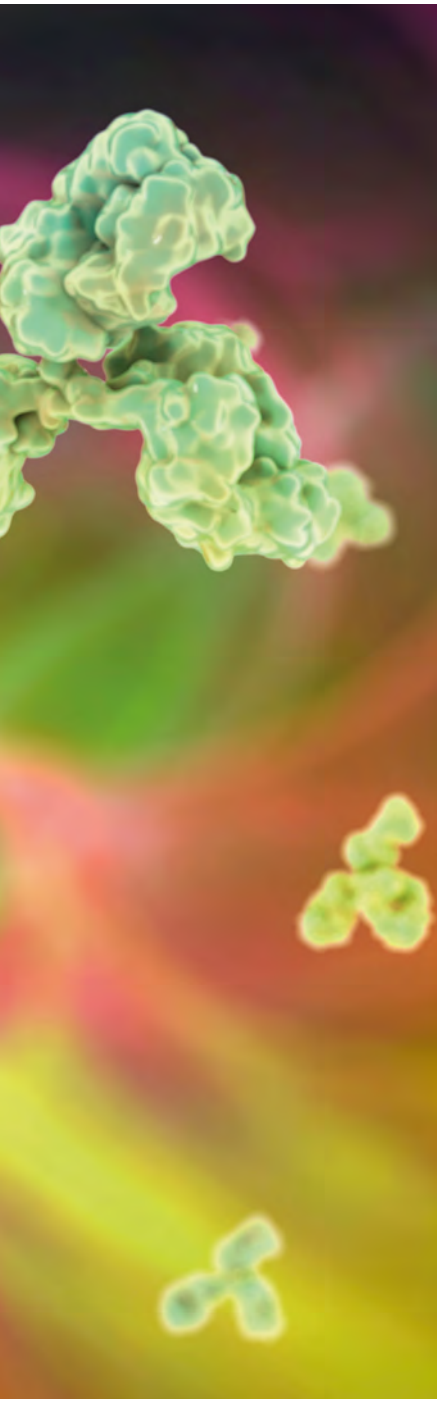
Multiplexed single-cell analysis enables simultaneous measurement of multiple properties – including secretion, antigen-specific binding, viability, and function – within a single run.

Cyto-Mine Chroma extends picodroplet screening through independent colour channels and multi-laser detection. These capabilities support more complex assays than single-parameter secretion screens, including FRET-based secretion assays and cell-binding assays in which antibody-secreting cells, target cells and bound antibody can be distinguished through different fluorescence signals.

The practical advantage is earlier characterisation. Researchers can move from asking whether a cell secretes an antibody to asking whether it secretes the right antibody, whether that antibody binds the intended target in a relevant assay context, and whether the selected cell can be recovered for downstream work.

The enabling architecture: Droplet-based microfluidics

Cyto-Mine Chroma leverages picodroplet microfluidics to



> **Antibody quality is rarely defined by one measurement**

encapsulate individual cells into miniaturised, isolated reaction chambers.

Within each droplet, secreted antibodies accumulate in a confined volume, assay reagents interact with secreted molecules, and multiple fluorescence readouts can be captured simultaneously.

This enables millions of parallel experiments, creating a direct link between cell identity and functional output. Each droplet becomes a contextual lens for understanding cellular behaviour at scale.

Because selected droplets can be sorted and dispensed into microplates, the workflow does not end at detection. It links screening directly to recovery, sequencing, culture or recombinant expression, helping preserve candidates that may otherwise be lost between workflow steps.

Insight at the extremes: Finding what matters most

The most valuable antibody candidates are often rare because they combine several desirable properties: they may be secreted by low frequency B cell population, bind a difficult antigen, show certain functional activity, and remain recoverable for downstream analysis. Traditional sequential workflows can miss these cells if they fail an early proxy assay or are diluted out before characterisation is complete.

Multiplexed platforms such as Cyto-Mine Chroma enable these rare cells to be identified and characterised in a single step, rather than through sequential filtering.

This shifts discovery logic from progressive narrowing to immediate identification of high-value candidates.

Real-world insight: Plasma cell discovery at scale

Recent peer-reviewed work by *Yu et al*. highlights how rapid B cell screening using Cyto-Mine translates into real-world discovery impact[1].

In this paper, Biointron Biological researchers demonstrated:

- Screening of **one to two million plasma cells per run**
- Recovery of **hundreds to thousands of unique antibody sequences within one week**
- Functional validation – including binding specificity – within **three to four weeks**

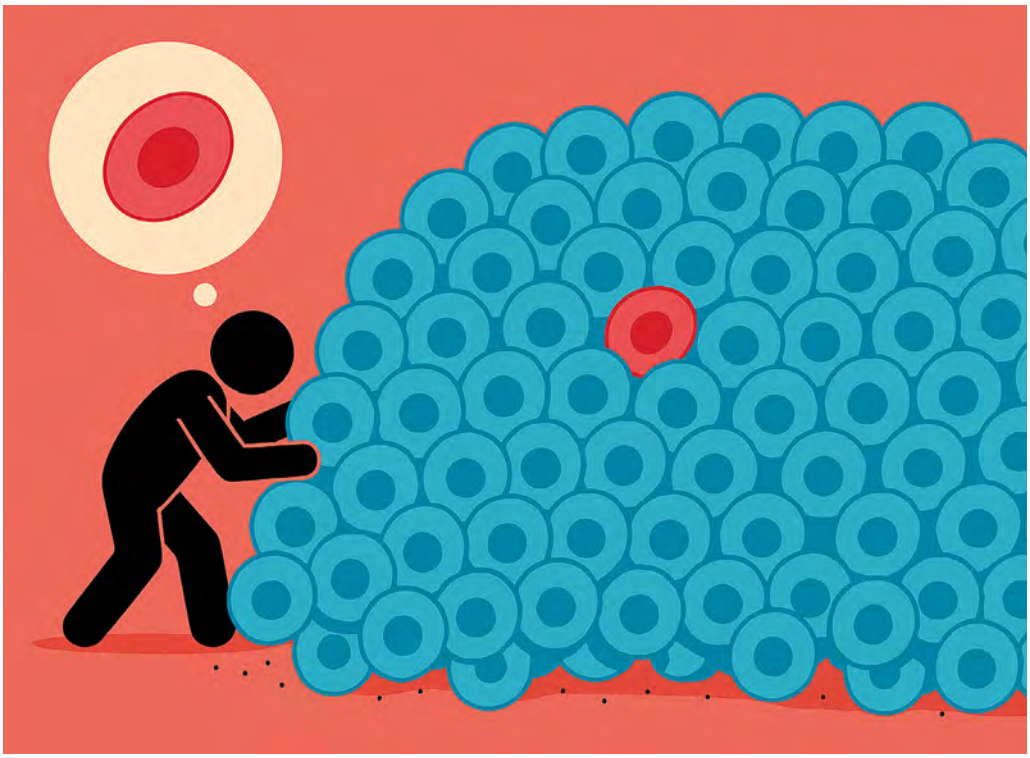
Their integrated workflow combines droplet screening, sequencing, and expression – compressing the path from **cell to functional antibody** into a single continuous process.

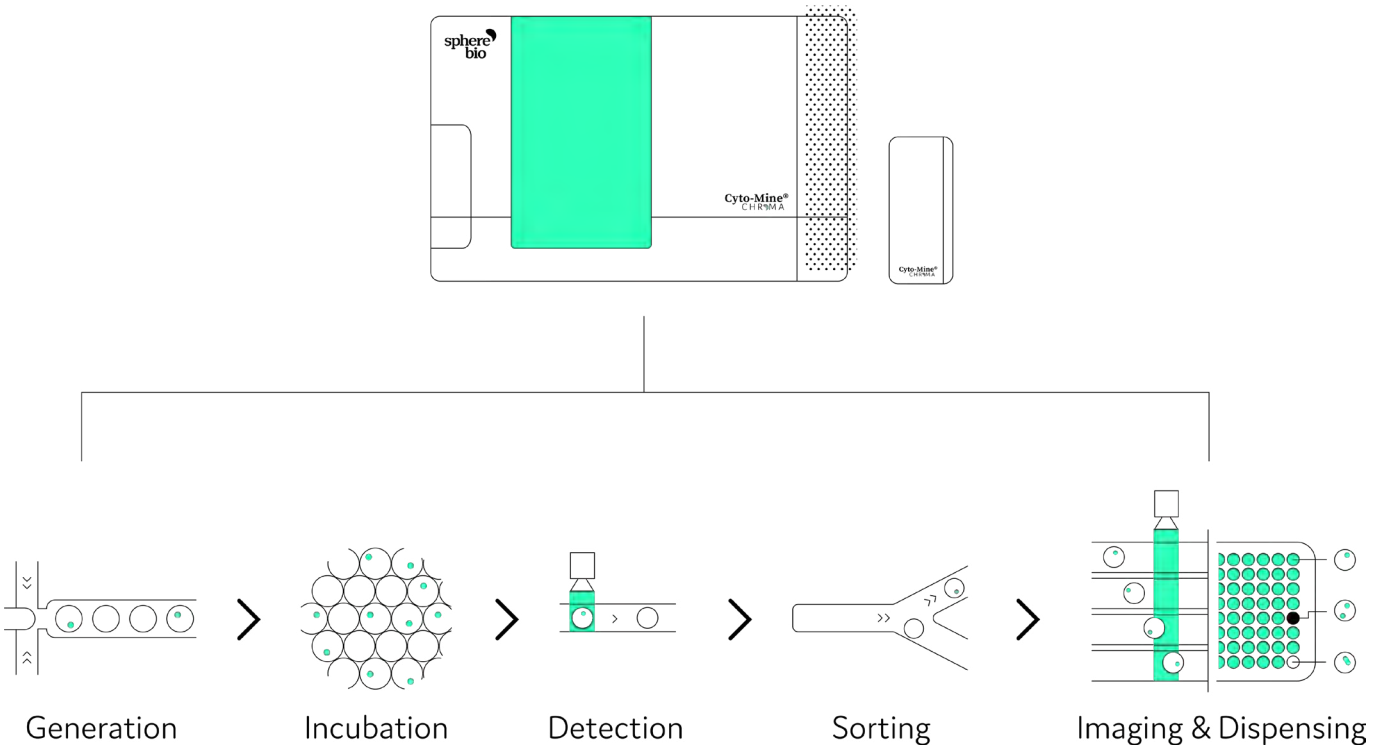
This example is important because plasma cells provide access to **high-affinity, naturally matured antibodies**, and can reveal diverse functional profiles – including both blocking and agonistic activities.

The broader implication is clear: multiplexed, high-throughput screening enables researchers to **access the full diversity of the immune repertoire – and act on it immediately**.

From sequential to integrated thinking

Traditional discovery follows sequential steps: first secretion, then binding, then function, stability, then developability. This structure





introduces delays, sample transfers and decision points that may remove valuable candidates too early.

Cyto-Mine Chroma enables these attributes to be assessed simultaneously, collapsing workflows into a unified process.

Discovery becomes a continuous, data-driven flow rather than a fragmented pipeline.

This reduces delays, minimses data loss, and strengthens decision-making earlier in the process.

Subtle signals, significant outcomes

Breakthrough antibodies are often defined by balanced performance across multiple characteristics not by dominance in a single parameter.

Multiplexed analysis enables these patterns to be detected early, helping researchers identify candidates that would otherwise be overlooked.

This marks a shift from identifying hits to selecting fully contextualised, high-quality leads.

Extending impact across the workflow

Earlier, higher-confidence selection improves outcomes across the pipeline.

With technologies such as Cyto-Mine Chroma, better early decisions lead to reduced attrition, faster development timelines, and more efficient resource use.

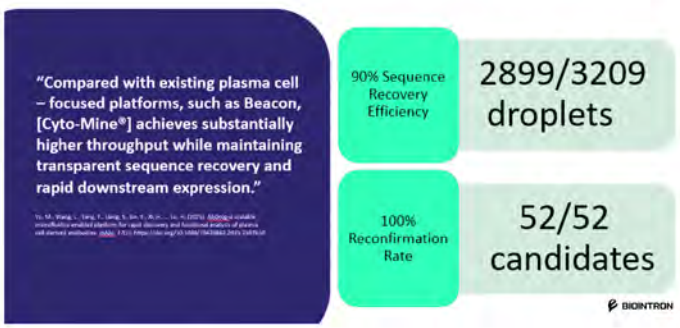
The benefits of multiplexing compound throughout the clone-to-candidate journey.

Toward multi-dimensional discovery

Antibody discovery is evolving toward integrated, high-content, and data-rich workflows. The future of rapid B cell screening will be defined by the ability to capture more information from scarce samples, preserve rare biological events and connect early phenotype to downstream sequence and function.

Platforms like Cyto-Mine Chroma are designed to support this shift – enabling scalable multiplexing, automation, and seamless workflow integration.
They allow researchers to interrogate millions of cells while retaining the resolution needed to identify the most promising ones.

This aligns with a broader shift toward understanding biology in high-resolution, multi-dimensional context.



Conclusion: A different kind of acceleration

Antibody discovery is no longer defined purely by speed – it is defined by quality of insight generated early enough to change the course of discovery.

Whether researchers are using rapid B cell screening or hybridoma technology in their antibody discovery workflow, multiplex-capable single-cell platforms such as Cyto-Mine Chroma enable researchers to see more, understand earlier, and act with confidence.

By redefining what can be known at the point of discovery, they transform the journey from clone to candidate.

In a landscape where the most valuable discoveries are hidden in rare cells, the ability to see clearly – and early – becomes the decisive advantage.

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