

Review

Single B cell technologies for monoclonal antibody discovery

Alessandro Pedrioli¹ and Annette Oxenius ^{1,*}

Monoclonal antibodies (mAbs) are often selected from antigen-specific single B cells derived from different hosts, which are notably short-lived in *ex vivo* culture conditions and hence, arduous to interrogate. The development of several new techniques and protocols has facilitated the isolation and retrieval of antibody-coding sequences of antigen-specific B cells by also leveraging miniaturization of reaction volumes. Alternatively, mAbs can be generated independently of antigen-specific B cells, comprising display technologies and, more recently, artificial intelligence-driven algorithms. Consequently, a considerable variety of techniques are used, raising the demand for better consolidation. In this review, we present and discuss the major techniques available to interrogate antigen-specific single B cells to isolate antigen-specific mAbs, including their main advantages and disadvantages.

mAbs: magic bullets in medicine

Highly specific mAbs functionally surpass **polyclonal antibodies (pAbs)** (see [Glossary](#)), which are a heterogeneous and undefined mixture of mAbs [1,2]. mAbs are broadly used as highly specific diagnostic tools, and as therapeutic modalities in the context of viral infections, tumors, autoimmune diseases, metabolic diseases, neurological diseases, and transplantation [3,4]. Particularly in the field of immuno-oncology, mAbs, and variants thereof, namely **single chain variable fragments (scFvs)**, **antibody–drug conjugates (ADCs)**, **bispecific antibodies**, etc. are gaining importance as leading therapeutic biologic drugs [4,5]. Since the USA FDA approval of Orthoclone OKT3 (anti-CD3 mAb) in 1986 for kidney transplantation rejection (the first therapeutic mAb), more than 100 mAbs have been approved by the FDA [6]. Furthermore, the current COVID-19 pandemic highlights how this class of biologics can be crucial in the context of a therapeutic setting and how important the rapid and efficient development of biologics can be [7]. Moreover, mAbs can potentially play key roles in prophylactic settings as well (passive immunization), especially when considering pathogens such as respiratory syncytial virus, for which effective vaccines are still unavailable [8].

Following the classical **hybridoma** technology (the first technology developed to isolate mAbs), many other methodologies have been developed and refined to improve or even radically change (e.g., **phage display technology**) the discovery of mAbs ([Box 1](#)) [3,9,10]. [Table 1](#) (Key table) lists some of the major published protocols that use primary B cells for the identification of antigen-specific antibodies, including their specific features, such as time expenditure, costs, ability to target difficult antigens, affinity of retrieved mAbs, ability to isolate **rare binders**, throughput, and ease of use.

In this Review, we focus on these most frequently used technologies based on single B cell retrieval to identify antigen-specific mAbs. This includes the hybridoma technology, single B cell cultures using conventional laboratory equipment, surface staining techniques, single B cell

Highlights

Monoclonal antibodies (mAbs) are among the most important type of biologic drugs on the pharmaceutical market, as well as for diagnostic purposes. Presently, more than 100 mAbs have been approved by the US FDA against a variety of diseases such as cancer, infectious diseases, autoimmune diseases, and neurological disorders.

Primary antigen-specific B cells are the main source for obtaining antigen-specific mAb sequences, particularly using human specimens such as peripheral blood mononuclear cells. Also, the humanization of mAbs derived from other species (e.g. mice, rats, and rabbits) has become easier and more efficient.

Currently, single B cell screening systems bear multiple advantages over other systems, such as display technologies. Particularly, the *in vivo* development of mAbs favors the safety profile and the overall developability. It also has reduced off-target binding to the human proteome.

Single B cell technologies have significantly evolved, becoming faster and higher throughput than before. Nonetheless, hybridoma technology, the first technique in this field, still represents an important methodology and is well known within the scientific community.

At present no gold standard exists in the field, relying on a broad variety of different single B cell systems for mAb discovery – each with its advantages and disadvantages.

¹Institute of Microbiology, ETH Zürich, Vladimir-Prelog-Weg 4, 8093 Zürich, Switzerland

*Correspondence:
aoxenius@micro.biol.ethz.ch
(A. Oxenius).



Box 1. The advantage of B cell mAb selection

From a clinical point of view, there are several advantages to using human B cell-derived mAbs over other systems, such as phage display. Because of their *in vivo* development and **affinity maturation**, they show a rather high specificity towards their targets, usually proteins, allowing a tailored response with low off-target binding to other host proteins [15]. Related to this first characteristic, they also usually bear low immunogenicity upon *in vivo* administration [115]. Thus, hybridoma-derived mAbs have had, on average, a better general **developability profile** (a crucial aspect, considered the 'Achilles heel' of most biologics) compared to mAbs derived from, for example, phage display libraries [116–120]. This is also due to their native post-translational modification profile, which decreases the possibility of aggregation *in vitro* and importantly, *in vivo* [121,122].

The theoretical size of the human B cell repertoire is between 10^{12} and 10^{18} different B cell clones (defined as the mathematical possibility of different V(D)J recombinations) [123]. However, in reality the number of different B cell clones within an individual is lower (around 1×10^7 – 2×10^7 different circulating B cell clonotypes) [124]. This might be due to several factors, such as the sample size of each donor used to estimate the clonal repertoire, out-of-frame mutations inserted during **V(D)J recombination**, and the formation of self-reactive BCRs which eventually undergo clonal deletion [123–125]. Yet, one must acknowledge that in display technologies, such as phage display, the repertoire size of naive B cell libraries is usually above 10^9 possible combinations, outpacing *de facto* the natural B cell repertoire size in a given sample [126,127]. This aspect can be partially compensated by the possibility of obtaining PBMC-derived B cells from many different healthy donors or patients.

An important aspect of retrieving antibody-coding sequences from (single) B cells is the reliable PCR-based amplification of VH and VL genes, their pairing, and sequencing. A variety of different degenerate primers/primer sets (usually for nested PCR) have been designed and are available for the most commonly analyzed species, especially humans and mice. In our experience, the primer sets published by von Boehmer *et al.* allow to consistently amplify murine Ig genes [51]. For human Ig genes, the primer sets published by Friedensohn *et al.* have also demonstrated good results [128].

cultures in miniaturized equipment, as well as single B cell replica methodologies and protocols based on high throughput genetic analysis of single B cells (Figures 1 and 2). With the aim of providing a sound overview that supports the selection of a suitable technology when embarking on mAb discovery and isolation, these technologies are explained at a technical level, followed by a discussion of their key advantages and disadvantages (Table 1). In a first section, the technologies are presented and discussed in the context of conventional cell cultures, while the second section comprises microfluidics-based technologies.

Conventional cell-culture-based technologies

The *ex vivo* survival of B cells is generally difficult to sustain, which poses a challenge for the identification of B cells expressing an antigen-specific antibody (immunoglobulin). To increase their lifespan, immortalization procedures have been developed, such as the fusion of primary B cells with immortalized myeloma cells (hybridoma technology), or immortalization of human B cells by Epstein–Barr virus (EBV) infection. Alternatively, B cell culture conditions have been refined to: (i) either maintain antibody-secreting B cells long enough in culture to screen their secreted antibodies for specificity or function; or (ii) culture times have been minimized by enrichment of antigen-specific B cells. In this section, we describe and discuss these technologies and provide a graphical illustration in Figure 1.

B cell immortalization technologies

The hybridoma technology is probably the best-known immortalization technique of mouse B cells and since its development, has revolutionized mAb discovery. The key principle of this technology is to immortalize short-lived **antibody-secreting cells** (ASCs) by fusion with myeloma cells, generating immortal clones whose supernatant can be screened for the presence of antigen-specific antibodies [11]. Most commonly, immunized mice are rechallenged with the antigen of interest and a few days later, spleen cells, containing ASCs, are used for the immortalization procedure (Box 2). A variety of additional protocols have been developed that use primary ASCs derived from other organs and compartments, such as bone marrow, lymph nodes, and

Glossary

Affinity maturation: process within germinal centers of secondary lymphoid organs; antibody-coding sequences are randomly mutated (mostly in their CDRs) and subjected to a selection process via which B cells expressing an antibody with increased antigen-affinity have a survival advantage.

Antigen-agnostic: screening performed without knowing in advance the antigen of interest; for example, entire cancer cells or viruses.

Antibody avidity: sum of noncovalent bond strength of various antibody paratopes towards a given molecule.

Antibody–drug conjugate: antibody with a payload (usually a cytotoxic drug) attached to it through a linker.

Antibody-secreting cell: any type of B cell able to secrete a certain amount of soluble antibody.

Antigen tetramer: complex of four molecules of interest (usually biotinylated proteins) usually displayed on a fluorescently labeled molecule of avidin.

B cell activating factor: cytokine belonging to the TNF family.

B cell receptor: membrane-bound form of the antibody.

Bispecific antibody: bears two different antigen specificities.

Broadly neutralizing antibody: bears high neutralization potency towards different pathogen strains/species.

Chimerization: molecular biology technique used to, for example, replace the constant part of an antibody without modifying the antigen-binding region.

Chemistry, manufacturing, and controls: comprises all steps needed during development and manufacturing of a drug to ensure safety and consistency.

Developability profile: physicochemical properties of a protein (e.g., antibody) such as thermal stability and tendency to aggregate.

Förster resonance energy transfer: energy transfer from a donor chromophore to an acceptor chromophore.

Germinal center B cells: subtype of mature B cells found within secondary follicles (GC) in secondary lymphoid organs.

Human anti-mouse antibody: response elicited by the administration of murine-derived antibodies to humans.

Hypoxanthine–aminopterin–thymidine medium: used during the first phases of hybridoma generation; it

peripheral blood mononuclear cells (PBMCs) [12]. The original protocol relied on PEG-mediated fusion of splenic short-lived murine ASCs, naturally expressing the enzyme **hypoxanthine–guanine phosphoribosyltransferase** (HGPRT⁺), with immortalized murine myeloma cells (HGPRT⁻) [1,13]. This protocol has been further developed to accommodate the use of ASCs derived from different species [11].

For selection of immortalized HGPRT⁺, ASC–myeloma fusions are placed in medium containing **hypoxanthine, aminopterin and thymidine** (HAT medium); a first single cell subcloning step is needed to generate monoclonal immortalized hybridoma cell lines whose supernatants are then screened for antigen-specific clones, and corresponding clones are subjected to further subcloning cycles (usually two or three) by limiting dilution to generate stringent monoclonality [14]. Confirmed antigen-specific clones are then expanded and utilized to produce the desired mAbs. Most of the first mAbs approved and currently on the market were generated by using a hybridoma technology, often followed by **chimerization** or **humanization** processes to overcome **human anti-mouse antibody** reactivity (HAMA, if hybridomas were raised from a murine host) as well as **in vitro affinity maturation** [15].

The whole protocol, excluding the immunization/infection of the mice, takes approximately 4–6 months [16]. In addition, the procedure has a low throughput due to the extremely low percentage of successfully fused ASC / myeloma cells – usually around one ASC out of 100 000, using the original PEG-based protocol [17]. This has posed an important bottleneck, making the antibody-discovery procedure lengthy [18,19]. Newer protocols accommodate several improvements, including some additional tissue culture measures such as the addition of cytokines and peritoneal macrophages to remove dead cells [20]. Some protocols also attempt to go beyond the PEG-based cell fusion step, implementing either electrofusion-based systems, or making use of fusogenic viruses such as Sendai virus or vesicular stomatitis virus to potentially increase the fusion efficiency between ASCs and myeloma cells, which represents one of the most relevant downsides of generating hybridomas [17,21,22].

Moreover, some hybridomas are low-yield antibody producers (or become so after numerous passages), rendering them not suitable for efficient use in GMP-grade bioreactors over long periods. This is partially due to the inherent chromosomal instability of a relatively high percentage of hybridomas [23,24]. Another issue is the competition between different hybridomas in the same culture upon PEG fusion, especially during the first steps of the protocol. In fact, some non- or low-producing clones may exhibit a faster growth rate, outcompeting high-secreting clones [25]. Thus, the initial subcloning step is usually performed in the shortest time possible (within 1 or 2 weeks), requiring later-on **high-throughput screening** of subcloned hybridoma candidates. Alternatively, for human B cells, immortalized B cells can be produced by using primary B cells immortalized with EBV, as discussed in the next section [26,27].

Collectively, the hybridoma technology bears several disadvantages, as discussed in the preceding text and summarized in Table 1, leading to decreased use of this technology. Despite that, the availability of soluble antibodies allows the screening of difficult antigens, as well as the functional screening (e.g., virus or toxin neutralization) with conventional laboratory equipment and expertise (Box 3). This is probably the main reason why the hybridoma technology is still used by some laboratories after almost half a century of its first description.

Memory B cell and ASC culture techniques

In the past two decades, some advances have been made in defining specific culture conditions for primary B cells without relying on the generation of hybridomas or EBV immortalization [28].

inhibits *de novo* DNA synthesis (through aminopterin) but allows the switch to the salvage pathway (through hypoxanthine and thymidine), promoting survival of cells that express HGPRT.

Hypoxanthine–guanine phosphoribosyltransferase

enzyme: needed for the generation of purine nucleotides through the purine salvage pathway.

High-throughput screening: typically, a phenotypic screening using at least hundreds of thousands of potential screening candidates, such as single B cells with different specificities.

Humanization: process beyond chimerization, whereby in addition to, for example, the constant region in an antibody, the original framework regions (FRs) of the antibody of interest are substituted with human FRs without compromising the CDRs responsible for the paratope-epitope binding.

Hybridoma: primary short-lived B cell fused with immortal myeloma cell.

In vitro affinity maturation: assay usually performed with display technologies, for example phage/yeast display; allows an increase in antigen-binding affinity ($K_d = K_{off}/K_{on}$) of a given antibody.

Memory B cells: antigen-experienced B cells carrying membrane-bound antibody (BCR); without any additional exogenous stimuli, they do not produce detectable quantities of secreted antibody.

Next-generation sequencing: technology allowing to sequence a mixture of different DNA molecules at once in a high-throughput fashion.

Polydimethylsiloxane: polymeric compound with which many microfluidic chips are made of.

Phage display: technique used to display libraries, for example, of VH–VL combinations on a filamentous phage containing the respective genomic information to detect antigen-specific VH–VL pairings.

Plasma B cells: terminally differentiated mature B cells almost completely lacking any membrane-bound antibody (depending on isotype and subtype), but secreting large amounts of antibodies.

Polyclonal antibodies: mixture of different antibodies usually extracted from the serum or ascites of immunized mice or convalescent sera of human patients.

Public clones: similar or identical (shared) antibody VH–VL sequences generated from different individuals.

These protocols mostly exploit commodity equipment with B cells being individually placed in 96 or 384 well plates through limiting dilution, enabling the interrogation of several hundreds of thousand ASCs, and up to 1 billion [29–32]. Tailored instruments specifically developed to screen ASCs and activated **memory B cells** are listed in a separated paragraph (single B cell screening methodologies).

Due to their fundamental importance in potential therapeutic use, several of these protocols have focused primarily on human B cells, namely memory B cells and ASCs, often without distinguishing a specific subset, even though usually PBMCs are used; therefore, these most likely comprise plasmablasts and not **plasma cells** (PCs). The significant increase in throughput by these novel B cell culturing systems, compared to the more limited throughput achieved with the conventional hybridoma technology, has allowed to retrieve e.g. the sequences of important, yet infrequent, **broadly neutralizing antibodies** (bnAbs) against HIV-1 [3].

Some of these studies have even been conducted in an **antigen-agnostic** manner; the supernatant of these single B cells has been used directly in virus neutralization assays (HIV-1 or SARS-CoV-1), without assessing beforehand, antigen specificity (i.e., antigen-binding by ELISA) (Box 3) [33–38].

Most human memory B cell cultures rely on a feeder layer expressing CD40 ligand (CD40L) in combination with cytokines such as IL-4, IL-10, and/or IL-21, or on immortalization by EBV infection in combination with TLR9 agonist such as CpG, and sometimes, cognate antigen [36,39–47]. Occasionally, restimulated EBV-immortalized human memory B cells have been used as fusion partners for myeloma cells to improve the generation of hybridomas (so-called EBV hybridomas) [26,27].

An alternative is the fluorescent foci method, in which secreted antibodies are detected from single plasma cells, which are placed on microscopic slides in a semiviscous medium together with antigen-coated beads and a fluorescently labeled secondary antibody. The secreted antibodies

Rare binders: extremely low percentage (<0.1%) of single B cells that are specific for a given antigen.

Reverse transcription: synthesizes cDNA molecules from mRNA molecules.

Single chain variable fragment antibody: short protein composed uniquely of VH and VL regions of an antibody, fused together, usually by a glycine-serine linker.

Somatic hypermutation: random mutational process occurring during germinal center reactions; cytosine is deaminated leading to a uracil via the enzyme activation-induced cytidine deaminase.

V(D)J recombination: developmental process of B and T cells. In B cells, variable (V), diversity (D, only in the heavy chain) and joining (J) gene segments are recombined together, giving rise to a mature antibody variable gene sequence: V(D)J sequence.

VH–VL pairing: natural pairing of the variable regions of recombined VH (variable heavy) and VL (variable light) chain loci in a specific antibody.

Virus-like particle: nonreplicating organic spheric structure resembling a virus; carries repetitive membrane-bound viral proteins on the surface.

Key table

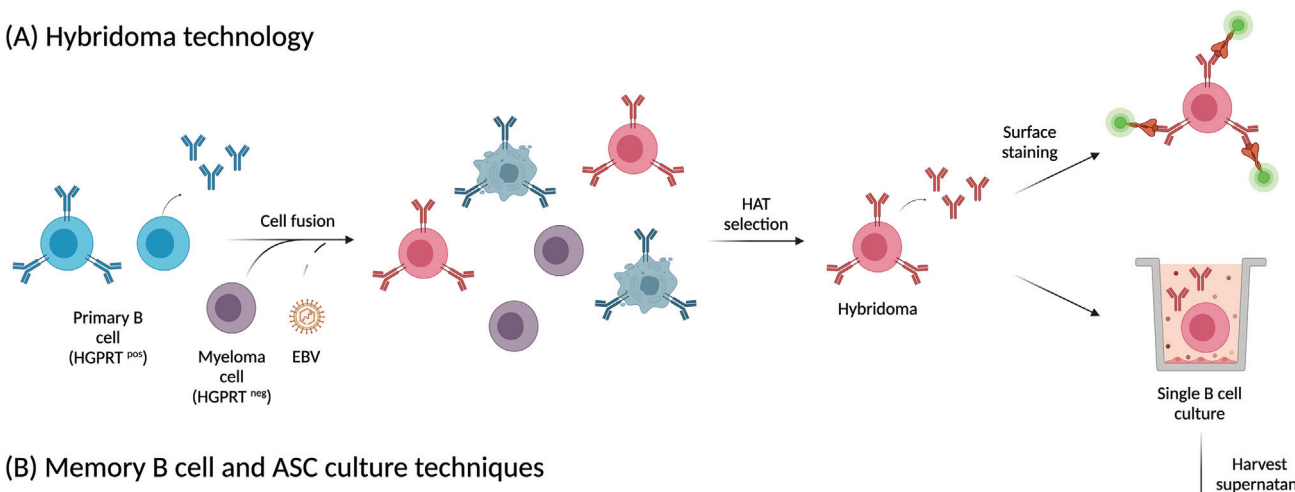
Table 1. Platforms used to discover antigen-specific mAbs from single B cells^{a,b}

Technology	Time	R&D Cost	Difficult antigens	Average affinity	Rare binders	Throughput	Ease
Hybridoma	Red	Green	Green	Green	Red	Red	Yellow
Memory B cell and ASC culture techniques	Green	Green	Green	Green	Yellow	Yellow	Green
Membrane-bound BCR staining of B cells with antigen bait	Green	Green	Yellow	Yellow	Green	Green	Green
Single B cell screening methodologies	Green	Red	Green	Green	Green	Green	Yellow
B cell replica methodologies	Yellow	Red	Green	Green	Green	Green	Red
Single B cell repertoire analysis and clonal expansion-guided identification	Red	Yellow	Green	Yellow	Red	Yellow	Yellow

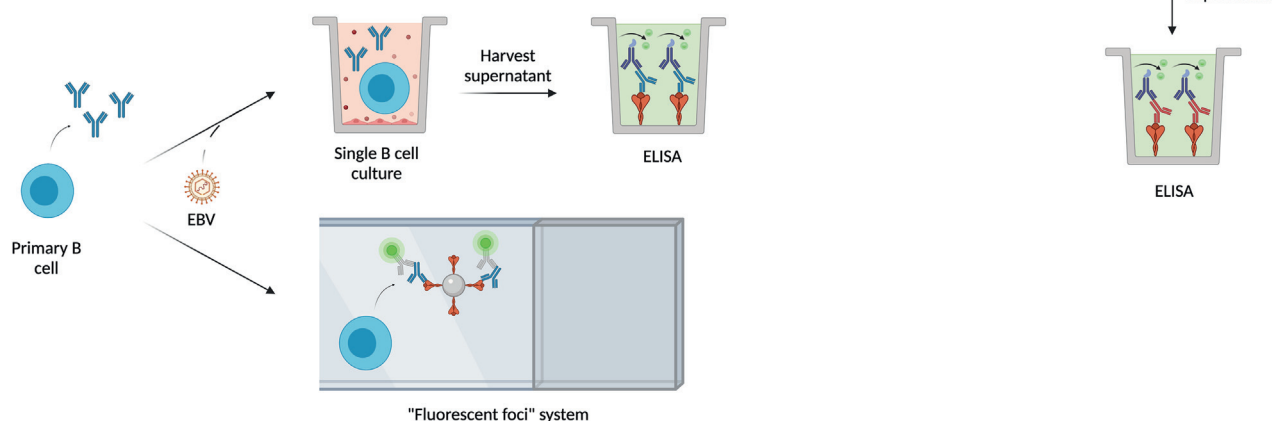
^aColor legend: green: good performance, yellow: medium performance, red: low performance

^bThe different techniques discussed in this review were compared by considering several relevant parameters such as time requirement, cost, possibility of screening difficult antigens (Box 3), chances of identifying rare binders, throughput, and ease of screening.

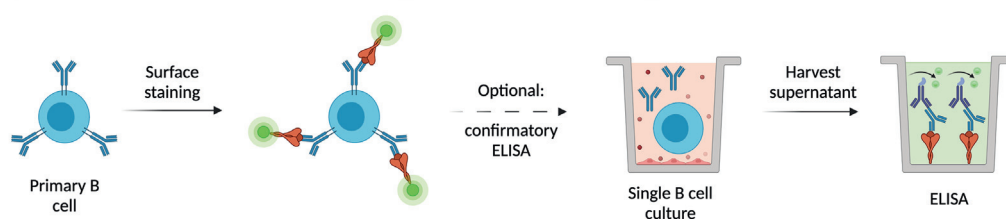
(A) Hybridoma technology



(B) Memory B cell and ASC culture techniques



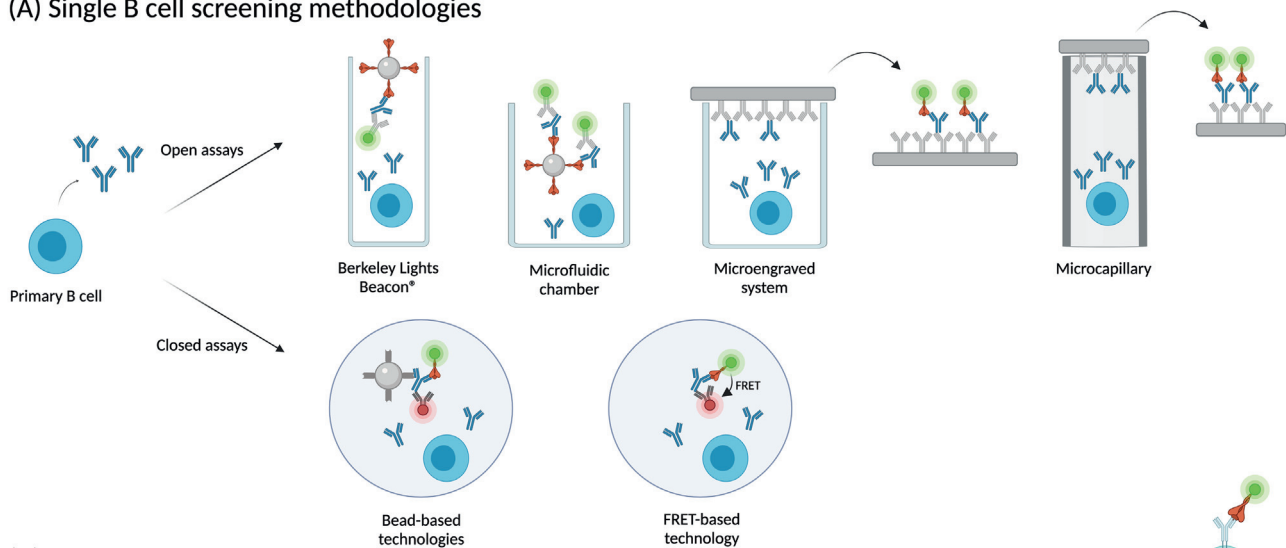
(C) Membrane bound BCR-staining of B cells with antigen bait



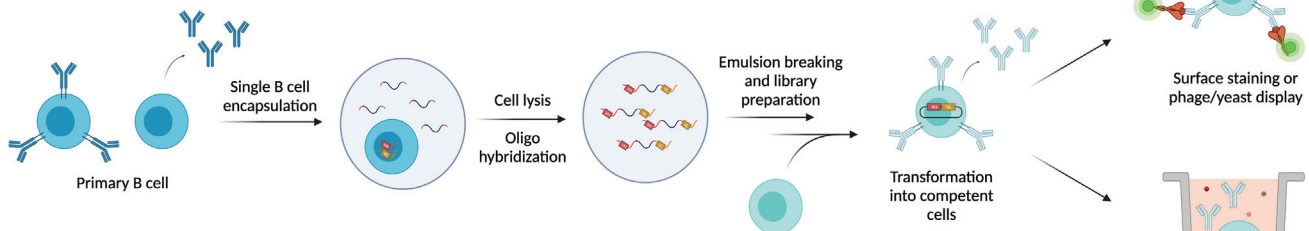
Trends in Immunology

Figure 1. Single B cell screening technologies using conventional cell culture equipment. (A) Hybridoma technology. Primary B cells (HGPRT^{pos}) are fused with myeloma cells (HGPRT^{neg}), giving rise to hybridomas. Upon selection with HAT medium, only fusions between primary B cells and myeloma cells will survive. Bulk hybridomas are then subjected to limiting dilution, generating monoclonal B cell hybridomas, and left to secrete antibodies which are then used to confirm antigen specificity. Alternatively, the BCR can be exploited in a conventional surface staining with fluorescently labeled antigen. (B) B cell culture. Primary B cells (typically ASCs) are individually placed in culture wells with tailored culture conditions, such as cytokines and a feeder cell layer. Additionally, EBV can be used to immortalize human B cells. The supernatant containing the secreted antibodies is then screened in a conventional ELISA assay using the antigen of interest. Alternatively, ASCs are seeded on a microscopy slide coated with a semiviscous medium and left to secrete antibodies which bind antigen-coated beads. These antibodies are then detected by fluorescently labeled secondary antibodies forming the fluorescent foci. (C) Memory B cell sorting. The BCR of a memory B cell is stained by binding to a fluorescently labeled antigen. The candidate memory B cells are then isolated and used for downstream processing (RT-PCR) or placed into culture to perform a confirmatory ELISA for antigen specificity. This figure was created using BioRender (<https://biorender.com>). Abbreviations: ASC, antibody-secreting cell; BCR, B cell receptor; EBV, Epstein-Barr virus; HAT medium, hypoxanthine, aminopterin and thymidine medium; HGPRT, hypoxanthine-guanine phosphoribosyltransferase.

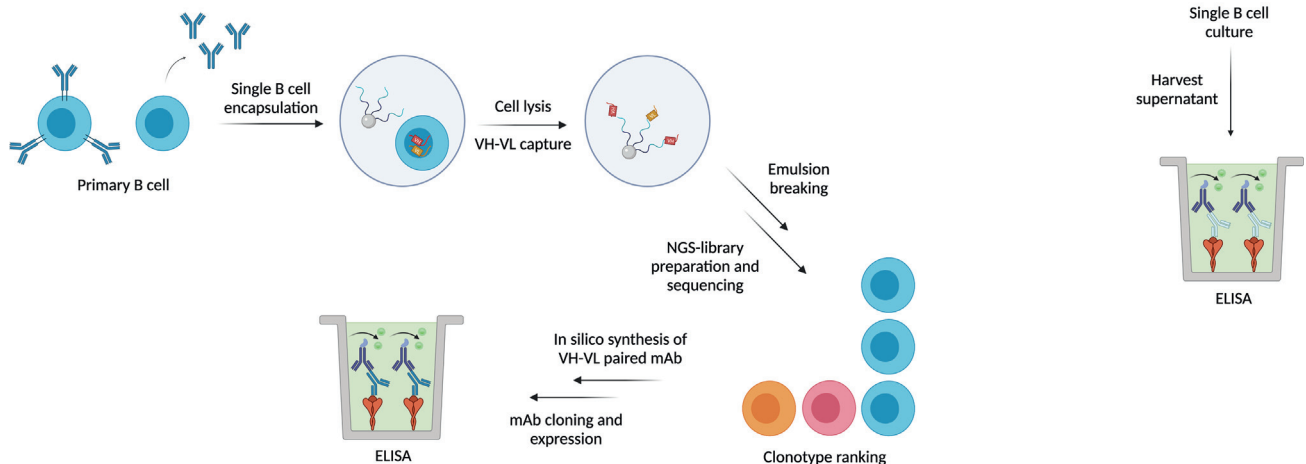
(A) Single B cell screening methodologies



(B) B cell replica



(C) Single B cell analysis and clonal-expansion guided identification



Trends in Immunology

Figure 2. Single B cell screening technologies using miniaturized equipment. (A) Single B cell screening methodologies. Usually, ASCs are used with these technologies, as all rely on secreted antibodies. Alternatively, activated memory B cells can also be used. We divided methodologies into open and closed assays. Among the open assays is the Beacon (Berkeley Lights) technology, the microfluidic-chamber, the microengraved systems, and the microcapillary system. Closed assays comprise all the methodologies relying on encapsulation of single cells in water-in-oil droplets. Both open and closed technologies mainly rely on the detection of antigen-specific antibodies via either colocalization of secreted antibodies and beads displaying antigen on their surface, or soluble antigen that is bound by anti-IgG-captured secreted antibodies either via colocalization of different fluorophores or via generation of a Förster resonance energy transfer (FRET) signal. (B) B cell replica. Primary B cells are encapsulated in a water-in-oil droplet and paired VH-VL sequences are amplified in one sequence using oligos containing complementary antibody

(Figure legend continued at the bottom of the next page.)

Box 2. Immunization protocol

This first step of immunizing host species with a specific antigen of interest is still common to many B cell screening systems, relying heavily on *in vivo* generated antigen-specific B cells from different species. Alternatively, B cells can be selected from healthy volunteers, individuals, or animals known to be seropositive for a specific antigen of interest. Different improvements of the immunization strategy, such as adjuvants used along with the antigen of interest and optimized immunization schedules, have been developed to elicit strong humoral responses, including those against difficult antigens such as membrane proteins, GPCRs, ion channels, and carbohydrates [131–133]. Usually, researchers still rely on the classical prime–boost strategy, using specific boosting schedules with respect to the number of boosts and time intervals between them. The antigen of interest used for immunization has to be carefully selected because the antigen must remain in the original conformation even upon administration (usually intraperitoneal administration.) [134]. This poses a first bottleneck, as many antigens of interest (e.g., ion channels and GPCRs), are not available in solution, and among those fulfilling this need, there is no certainty that the protein of interest will elicit a humoral response against the epitopes of interest. For example, upon administration, the radical change in the surrounding medium (e.g., mouse whole blood instead of PBS) might lead to a conformational change of the protein of interest, potentially exposing different epitopes. This might lead to the activation of B cells secreting antibodies that specifically target these epitopes being exposed in a non-natural environment, which are not necessarily accessible on the same protein when in their natural environment, such as in the plasma membrane or in solution (e.g., PBS). Furthermore, some antigens might be toxic and therefore compromise the animal's health, thereby posing a hurdle for generating an appropriate humoral response. To overcome these obstacles, novel strategies are exploiting the possibility of performing genetic immunizations with plasmids encoding the antigen of interest, and/or by multiplexing the whole immunization process using several different antigens at once, or within a short time period, thereby relying on a smaller number of animals [135–138].

are confined to the proximity of the ASCs by low diffusion in the semiviscous medium, and ASCs of interest are retrieved using a micromanipulator [32]. Unlike the method described previously, this latter method allows the screening of a large number of single B cells as there is no need to create a physical separation between different ASCs by using culture wells.

Methodologies enabling the study of murine antigen-specific B cells have also been developed, especially regarding *in vitro* derived plasma cells and memory B cells. The Nojima culture describes the generation of *in vitro*-induced **germinal center B cells** (iGC B cells) from murine naïve B cells, generated with a specifically engineered feeder cell line termed 40LB, which constitutively expresses CD40 ligand (CD154) and **B cell activating factor** (BAFF), together with exogenous recombinant murine IL-4 and IL-21, thereby mimicking *in vivo* conditions [48,49]. This culture method allows sustained viability of an *ex vivo* culture of murine ASCs [50]. In general, the above-mentioned B cell screening protocols are widely used, due to their medium to high throughput, simplicity, and limited costs (Table 1). Furthermore, as the screening makes use of the secreted antibodies, there is a substantial advantage in terms of screenable targets, ranging from soluble proteins to entire cells or viruses, as further discussed in Box 3.

Membrane-bound BCR staining of B cells with antigen bait

To enrich antigen-specific memory B cells, their membrane-bound antibodies (**B cell receptor**; BCR) can be used to bind an antigen of interest that is coupled to a fluorescent dye, followed by sorting of stained memory B cells by FACS. There are several detailed protocols explaining the slightly different procedures for isolation of antigen-specific B cells for various species, but they

constant region sequences. In order to do so, cells are lysed and both the *VH* and *VL* single B cell-mRNAs anneal to the encapsulated oligos. Upon emulsion breaking, the library is then prepared through RT-PCR and cloned into suitable expression vectors. This library is then transformed into expression cells such as yeast, phage, or mammalian cells. The obtained cell population is then further used in conventional single cell screenings such as surface staining with fluorescently labeled antigens or ELISA of antibody secreted from these single engineered host cells. (C) Single B cell analysis (*VH*–*VL* paired). Primary B cells are encapsulated together with beads bearing barcoded-oligos and subsequently lysed, allowing the oligos to anneal with single-cell total mRNAs. Upon emulsion breaking, these oligos are then amplified and prepared for next-generation sequencing. After data analysis the antibody clones are selected mainly based on clonotype expansion (particular *VH*–*VL* pairing), but also potentially considering other characteristics (see main text). Antibody sequences of the most expanded clones (or taking into account additional features) are then synthesized, cloned into suitable plasmids, and recombinant monoclonal antibodies are generated; these are then tested with conventional techniques, for example, ELISA. This figure was created using BioRender (<https://biorender.com>). Abbreviation: mAb, monoclonal antibody.

Box 3. Soluble versus membrane-bound antigens

Antibodies from single B cells are present in two forms: membrane-bound and soluble, depending on the specific subset of B cells analyzed. This dichotomy is present on the antigen side as well (soluble versus membrane bound), forming a two-per-two matrix scenario (Figure 1). Soluble antibodies can be promptly screened against both soluble and membrane-bound antigens, but they typically lose the genotype–phenotype link because of their diffusion in the medium if no physical constraint such as a plate well or droplet is in place. By contrast, membrane-bound antibodies retain this latter fundamental link, but their screening is largely limited to labeled soluble antigens or to engineered VLP/virions.

As described for many screening techniques, the need of having a soluble target antigen has some potential drawbacks, especially because many challenging antigens (e.g., complex viral glycoproteins), are notoriously difficult to produce and to engineer in a native-like soluble oligomeric form, and ideally, in a stable prefusion conformation [3,139]. One of the most notable examples is the pre-fusion Env trimer of HIV-1. It took approximately 30 years from the discovery of HIV to generating a near-native soluble conformation of Env (termed BG505 SOSIP) – able to elicit a potent humoral response upon immunization in animal models [140].

Moreover, a potential disadvantage is that by over-crosslinking the antigen of interest during the process of fluorophore conjugation or when generating antigen tetramers using biotin, there is a certain risk of antigen- or antigen-specific B cell aggregation. In line with this observation, there is also an inherent risk of blocking some interesting epitopes by steric hindrance, especially the ones bearing a substantial amount of primary amines, conventionally exploited by N-hydroxysuccinimide chemistry-based crosslinking protocols [63].

Finally, to favor the discovery of antibodies against difficult/unknown targets in these B cell interrogation assays, an interesting approach may be to perform an antigen-agnostic screening, only possible to date with soluble B cell-secreted antibodies [111].

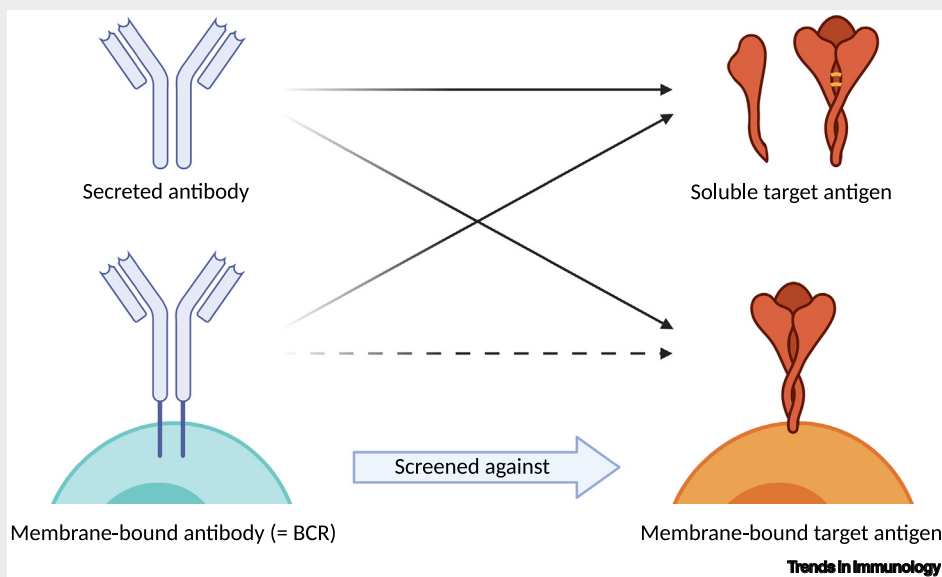


Figure 1. Possibilities and limitations of using membrane-bound and soluble antibodies or antigens. Soluble antibody (top left) secreted from antibody-secreting cells can be readily used to screen against soluble as well as membrane-bound antigens of interest (right column). By contrast, membrane-bound antibody (bottom left) can easily detect soluble antigens (e.g., memory B cell screening), but it is technically challenging to screen for membrane-bound antigens (depicted as a broken line), unless laborious techniques such as engineered virus-like particles are used. Additionally, many antigens are difficult to express in a soluble form in their native conformation (e.g., trimer), and they often require further structural engineering [111]. Abbreviation: BCR, B cell receptor.

all rely on the use of a fluorescently labeled antigen [including virions and **virus-like particles** (VLPs)], or alternatively **antigen tetramers** (improving **antibody avidity**), antigen-coated beads, or antigen-nucleotide tags, for further high-throughput sequencing [51–60].

Generally, during the staining procedure, the labeled antigen of interest is used together with an irrelevant antigen that is labeled with another fluorophore to discriminate specific from nonspecific binders, in combination with antibodies for (memory) B cell-specific surface markers [61]. Alternatively, it is technically possible to fix and permeabilize cells in order to stain cytoplasmic antibodies, although this method negatively affects mRNA integrity [62].

Regardless of the identification protocol, putative antigen-specific single B cells are then either directly sorted into lysing buffer and **reverse-transcription** (RT) reagents, or placed *ex vivo* in single cell cultures with activating conditions (such as CD40L) for further downstream analysis of the secreted antibodies (confirmatory ELISA), followed by identification of the antibody-encoding variable heavy (*VH*) and light chain (*VL*) gene sequences through conventional mRNA purification, cDNA transcription, and sequencing [60].

Other than the need to use soluble antigens (Box 3), another caveat of this method is the potential false-positive binding (or stickiness) of B cells to fluorophores, streptavidin, and antigen purification tags (His-Tag, FLAG Tag, MBP, etc.), erroneously selecting some B cell populations [63]. Most of these false-positive events are excluded by using a dual labeling system of the same antigen with different fluorophores, and specifically selecting the double-stained B cell population [64,65].

This is one of the most used systems to isolate antigen-specific memory B cells, having several advantages such as high-throughput, low cost, and simplicity (Table 1). However, relying exclusively on isolating memory B cells expressing membrane-bound antibody might lead to some caveats, such as neglecting the selection of plasma cells, which are actually producing the circulating antibodies (Box 4).

Microfluidic-based technologies

Beyond the use of conventional laboratory equipment such as cell culture plates and microscopy slides for the interrogation of antigen-specific B cells, recently developed techniques use special miniaturized platforms based on microfluidic technologies [66,67]. This spatial confinement of individual B cells has further improved not only the *ex vivo* culture *per se*, but allowed the identification of individual antigen-specific B cells in a high-throughput manner within a short time frame (Figure 2).

Single B cell screening methodologies

In terms of using *ex vivo* B cell cultures, the key advantages of decreasing the assay volume consist mainly of time and throughput, as smaller test volumes substantially accelerate the time it takes to obtain a critical concentration of antibodies that are required for measuring antigen specificity [63,68–70]. Short culture conditions are particularly important for bone marrow-derived plasma cells that are known to have limited *ex vivo* survival (Box 4), jeopardizing antigen screening in long-term conventional cell culture conditions, as well as subsequent mRNA *VH–VL* retrieval upon successful screening due to compromised viability [71,72].

The semiautomated systems supporting miniaturized screenings can be subdivided into open assays, allowing for prompt manipulation of cells and medium, and closed assays, in which cell manipulation can be performed only by breaking the encapsulating reaction compartment (Figure 2). The category of open assays includes the Berkeley Lights Beacon, the microfluidic chambers, the microengraved systems, and the microcapillary array. By contrast, generally all microfluidics-based systems using water-in-oil droplets can be listed in the category of closed assays.

Box 4. Choice of B cell subsets

mAbs can be technically isolated and generated from every type of naïve and antigen-experienced B cell compartment, as they all carry V(D)J rearranged Ig genes [129]. In practice, however, mAbs are usually generated from four main types of B cells, namely GC B cells (the least used B cell subset), plasmablasts (short-lived plasma cells), memory B cells, and plasma cells (long-lived plasma cells) [61]. The choice of which subtype of B cell to use is largely dependent on two main factors: B cell subset availability and the methodologies available to interrogate the specific B cell compartments.

In humans, the most-used B cell subsets are memory B cells or antibody-secreting cells (particularly circulating short-lived plasmablasts), readily available in the PBMCs of healthy donors or patients. Human GC B cells and long-lived plasma cells are usually accessible only through complicated biopsies, and therefore, not the primary choice (see Table 1 in main text) [28]. Generally, the choice of patients or donors whose B cells will be selected for the antibody discovery project is dictated by the serum content of polyclonal antibodies and their quality (e.g., neutralization potency). Aside from humans, in rodents or other species (including humanized mice), the B cell subset availability does not pose a problem, as technically all B cell subsets can be easily retrieved (Table 1). Moreover, the use of transgenic mice, such as mice with human Ig loci, allows to elicit a robust human-like humoral response against human self-antigens [130].

Depending on the used strategy, antibody discovery can be essentially divided into two main approaches: analysis of membrane-bound BCR and interrogation of the secreted antibody from various B cells subsets [68]. The former strategy is usually the preferred one, being fast, easy, and high throughput. The antigen of interest must be known *a priori* and be soluble in a physiological buffer such as PBS [92]. The latter strategy, relying on the analysis of B cell-secreted antibody molecules, allows for the discovery of potential new targets, and can be combined with a variety of antigens, not only soluble but also expressed as membrane-bound proteins in target cells or cell lysates (see Box 3).

The Beacon technology places several thousands of individual ASCs or activated memory B cells into NanoPen chambers on a tailored culture chip (ranging from 740 pL to 1.7 nL in volume), in which they secrete antibodies into the supernatant [73]. For the detection of antigen-specific antibodies, antigen-coated beads are placed right above the NanoPen, surrounded by fluorescently labeled secondary antibodies. Here, secreted antibody binding to the antigen-coated beads is detected by the secondary fluorescent anti-IgG antibody, generating a fluorescent bloom. The screening phase takes usually only a few minutes to up to several hours, followed by specific single B cell retrieval using a structured light-based system termed Opto Electro Positioning (OEP) [73,74].

A similar method in a microfluidic chamber relies on a micro-engraved **polydimethylsiloxane** (PDMS) support, in which beads coated with the antigen of interest are added to the microwell containing the single B cell. Upon secretion of antigen-specific antibody by the ASC, and binding of a secondary fluorescently labeled antibody, there is spatial colocalization of the antibody-secreting B cell and the antigen-labeled bead, allowing the positive identification of microwells that host antigen-specific ACSs [75].

Another miniaturized technology includes a microengraved system, in which microchambers of 0.1–1.0 nL volume are printed on a PDMS chip, and ASCs are seeded into these microchambers via limiting dilution [76–79]. For the identification of single ASCs of interest, the system uses a precoated PDMS/glass chip with the antigen of interest (original protocol) which is ‘printed’ by the secreted antibodies and detected with a secondary labeled antibody. Alternatively, anti-IgG capture antibodies are placed right above the microchambers and labeled antigen is used, creating a halo around the well containing the single ASC of interest [77]. In both cases, there is a need for a special micromanipulator to retrieve the single ASC of interest.

Functionally comparable to the microengraved system, another type of open assay is the microcapillary array which allows higher throughput compared to the methods mentioned in the preceding text [80]. This method allows to screen millions of individual B cells [80]. The higher throughput is achieved by combining together various 40 µm porous glass fibers instead of printing

or engraving on a PDMS chip (regarding the above-mentioned systems). Like the previous method, the identification of an antigen-specific single ASC upon antibody secretion is mediated via a removable chip coated with the antigen of interest. The subsequent retrieval of the single ASC of interest occurs through a flow of nitrogen in the capillary containing the single B cell of interest, subsequently placing it in a conventional 384 well plate for further *VH–VL* retrieval [80].

Among the fully closed assays are the microfluidics-based systems, in which aqueous droplets are embedded in an oil-based medium, forming a water-in-oil emulsion (Figure 2) [81–88]. The technical principle behind these different microfluidic systems is comparable to the microfluidic chamber system, as they also rely on the colocalization of single antigen-specific ASC-secreted antibodies and a bead precoated with the antigen of interest, as well as a capture antibody for the ASC-secreted antibody. An exception in this regard is the Sphere Fluidics Cyto-Mine technology, which analyses the specificity of single ASC secreted antibodies by a **Förster resonance energy transfer** (FRET) signal, established by a soluble antigen of interest labeled with a donor probe and an anti-IgG protein labeled with an acceptor probe (detecting the ASC-secreted antigen-bound antibody) [89].

Upon identification of a target-specific single B cell, the subsequent retrieval of genetic information of the respective antibodies usually relies on coencapsulation of the ASC with individually barcoded beads displaying oligos with complementary *VH–VL* regions (to which single ASC-Ig mRNA can anneal), followed by lysis, library preparation, and **next-generation sequencing** (NGS) [81]. Alternatively, droplets of interest might be individually sorted directly within microfluidic chips, or via generation of a double emulsion followed by FACS; droplets are subsequently lysed (emulsion break) and further processed.

Similar to many other single B cell screening systems, there is a need to know *a priori* the target of interest (Box 3). Of note, it is possible for almost all the above-mentioned systems (except for the microengraved system and microcapillary array) to include not only antigen-coated beads, but also suspension cells that are engineered to display a surface antigen of interest (Box 3) [90,91]. However, besides the target-based approach, none of these systems have been shown to accommodate a functional assay yet (e.g., neutralization assay), which would ideally allow antigen-agnostic B cell interrogation for function [92].

Collectively, the main advantages of these systems are the high-/ultra-throughput and speed, and the main drawbacks of these systems lie in the cost and in the expertise needed to use them (Table 1).

B cell replica methodologies

The so-called B cell replica method aims to combine natively paired *VH–VL* single cell BCR^{neg} ASC-derived sequences (notably difficult to interrogate being short lived) with the high throughput and simplicity of the cell surface BCR^{pos} cell screening systems.

The essential idea is to conceptually invert the screening part and the cloning and expression part. Indeed, in all the above-mentioned systems, the screening against an antigen of interest occurs before the actual retrieval of Ig genes of the antigen-specific single B cell or hybridoma. In the B cell replica concept, the idea is to first clone and express (by displaying on the surface of permissive yeast/phage or mammalian cells) a certain single B cell-derived library (natively paired *VH–VL* derived from, e.g., immunized mice) and then, in a second phase, interrogate this library against an antigen of interest (Figure 2) [93].

With the advent of picoliter wells in PDMS or microfluidics techniques (instead of traditional cultures in 96- and 384-well plates), single cells can be encapsulated in an ultra-high-throughput fashion [69,94–96]. Conventionally, such methodologies use individual ASCs (i.e., plasmablasts and plasma cells; [Box 4](#)) which are coencapsulated with lysis reagents, RT master mix and, and a carefully designed oligonucleotide bridging the *VH* and the *VL* mRNAs by hybridization (**VH–VL pairing**). This latter element includes an in-frame sequence, coding for e.g. a glycine-serine linker, for subsequent display of the natively paired VH–VL sequence as scFv [93,97]. Upon lysis and RT, the water-in-oil emulsion is broken and the formed, natively paired VH–VL library is usually amplified further via PCR, allowing the addition of other genetic elements on both 5' and 3' ends, such as a transmembrane domain, or CRISPR-Cas9 overhangs. This complete library can then be displayed on the surface of yeast/phage cells or mammalian cells [93,97]. In this latter approach, the end product can be engineered in hybridoma/mammalian cells, therefore mimicking a natural bulk memory B cell population and making use of both surface-bound as well as secreted antibodies for antigen-specificity screening [98,99]. The first proof-of-principle use of this method allowed the isolation of anti-ricin and anti-influenza virus HA antibodies, confirming the technical feasibility of such an approach [93,100]. This methodology is an elegant solution, enabling the interrogation of natively paired VH–VL antibodies encoding genes of short-lived ASCs in the context of a long-lived, and simple to handle host cell line ([Box 4](#)). The major drawback is the technical complexity of the system, making this technology amenable for a restricted number of research laboratories ([Table 1](#)).

Single B cell repertoire analysis and clonal expansion-guided identification

A rather novel methodology to identify antigen-specific B cells makes use of the recently developed single cell transcriptomic techniques, such as the 10x Chromium, or any system using beads carrying barcoded-oligos, such as Drop-Seq [101]. The concept is based on the fact that upon immunization or infection, among the most expanded clones (defined as a unique homologous combination of natively paired VH–VL), there is a higher prevalence of antigen-specific B cells [102]. Using conventional bulk Ig NGS techniques would be unable to directly deliver the genetic information of native antigen-specific monoclonal antibodies, because of the technical inability to retrieve natively paired individual VH–VL sequences. Despite this latter limitation, a first proof-of-principle study demonstrated the concept of antigen-specific BCR mining by using B cell sequencing data (not natively paired VH–VL sequences), analyzing properties such as clonal expansion, **public clones**, and mutational burden [102]. The concept evolved further and a first panel of Ebola-virus-specific mAbs was identified based on the analysis of the most expanded clones upon Ebola VLP-based immunization of BALB/c mice and retrieval of natively paired VH–VL sequences using the picoliter well in a PDMS chip hosting individual B cells [94,103]. The most expanded unique VH–VL sequences were chosen as candidates and then synthesized, cloned into suitable mammalian expression vectors, and expressed as recombinant mAbs to corroborate their antigen specificity ([Figure 2](#)). Several other protocols have been reported since, mostly using single human B cells sequenced after microfluidic-based encapsulation for the discovery of mAbs against influenza virus, dengue virus, SARS-CoV-2, ovalbumin, and allergen-specific IgE [104–109]. Some of these protocols not only consider the clonal frequency of different clones, but also the frequency of **somatic-hypermutations** (SHMs) and potentially, other antigen-specific features; for example, focusing on already known antigen-specific VH–VL combinations, or specific IgG isotypes [104,105]. Technically, the basic concept moves away from the B cell replica methodology, as there is actually no physical copy of the B cell repertoire in permissive yeast/phage or mammalian cells; rather, it relies on the recombinant expression of the most likely candidate paired VH–VL genes, and the subsequent testing of the specificity of the respective antibodies. Unfortunately, this is a rather long procedure, considering that from the

B cell sorting to the final antigen specificity assay (e.g., through ELISA) of the candidate mAb, one should account for at least 2 months invested. Moreover, it involves several expensive and complicated steps, such as single cell encapsulation chips, bioinformatic analysis, and the production of numerous synthetic genes. Finally, because of the inherent nature of the system, that is, mainly analysis of the most expanded clones, the discovery of rare clones binding to a given antigen is seriously hampered, posing a bottleneck for the discovery of new VH–VL combinations with potentially interesting features (Table 1).

It might, however, be an interesting approach for discovering antibodies against undefined targets (target-agnostic), as the actual testing of the antigen-specificity of the mAb can be performed via ELISA, FACS, and also via *in vitro* neutralization assays (Box 3).

Concluding remarks

Despite the advent of many *in vitro* display technologies, the development of mAbs also relies on *ex vivo* single B cell interrogation (Box 1) [22,110]. Among the plethora of techniques enabling this process, the hybridoma technology is still used in new discovery pipelines because it is easy, cheap, and familiar to many scientists (Table 1). The main problems lie on the traditionally difficult-to-overcome mindset of having to adapt to new techniques, and the technical difficulty and execution of the new protocols (if described at all), as well as the price tag of the new instruments, making these methodologies out of reach for many laboratories (Table 1) [110].

Despite their difficult implementation, major advances are provided by the emerging new technologies for the interrogation of single B cells using high-throughput screening approaches, especially for the identification of mAbs that are specific for ‘high-hanging fruit’ antigens such as GPCRs, ion channels, and intracellular targets (Box 3) [92,111]. In addition to high-throughput systems, it will be crucial to implement efficient functional screening approaches, including for viruses and toxins, as well as for protein neutralization or receptor blockade (see Outstanding questions).

Furthermore, it is likely that when (and not if) artificial intelligence (AI)-based solutions are readily available, many of the antibody-discovery campaigns might be conducted almost completely *in silico*, with the important exception of the ensuing *in vitro* and *in vivo* validation. Especially in the last 2 years, major advances have been made in the field of AI-based *in silico* prediction of protein structures, which might allow to more precisely predict the structural basis of antibody–antigen interactions in the near future, without the need of wet-laboratory techniques *a priori* [112]. However, we expect that further advancement of single B cell technologies will still remain highly significant in mAb discovery; indeed, research focused on the isolation of natively paired VH–VL mAbs or on target-agnostic screenings remain fundamental research areas in immunology [113,114].

Acknowledgments

We are grateful for helpful input from members of the Oxenius laboratory. This work was supported by the Swiss National Science Foundation (grant nr. 310030_166078 and 310030B_185374).

Declaration of interests

No interests are declared.

References

1. Kohler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495–497
2. Lipman, N.S. et al. (2005) Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. *ILAR J.* 46, 258–268

Outstanding questions

Will it be possible to implement functional readouts (particularly antibody neutralization assays) with the novel single B cell technologies developed in the last decade? Can these dramatically increase the functional throughput?

Can we shortlist the antigen-specific antibodies that already display superior developability profiles during the early discovery phase? Developability profiles indeed represent an interesting feature: currently a given percentage of antigen-specific antibodies face serious **chemistry, manufacturing, and controls** (CMC)-related issues after lead discovery, hampering their use in clinical trials and ultimately, their development in the market. Providing a shortlist of specific mAbs could make the whole development process more efficient overall.

When and to what extent can AI-driven technologies allow a more efficient development of mAbs completely *in silico*? Can mAbs generated in this manner harbor comparable or even superior biological characteristics than single B cell-derived mAbs?

Under which specific conditions can antigen-specific mAbs be more efficiently developed to benefit preventive and therapeutic medical applications?

3. Wilson, P.C. and Andrews, S.F. (2012) Tools to therapeutically harness the human antibody response. *Nat. Rev. Immunol.* 12, 709–719
4. Weiner, G.J. (2015) Building better monoclonal antibody-based therapeutics. *Nat. Rev. Cancer* 15, 361–370
5. Walsh, G. (2018) Biopharmaceutical benchmarks 2018. *Nat. Biotechnol.* 36, 1136–1145
6. Mullard, A. (2021) FDA approves 100th monoclonal antibody product. *Nat. Rev. Drug Discov.* 20, 491–495
7. Taylor, P.C. et al. (2021) Neutralizing monoclonal antibodies for treatment of COVID-19. *Nat. Rev. Immunol.* 21, 382–393
8. Slifka, M.K. and Amanna, I.J. (2018) Passive immunization. *Plotkin's Vaccines* 84–95.e10
9. Mondon, P. et al. (2008) Human antibody libraries: a race to engineer and explore a larger diversity. *Front. Biosci.* 13, 1117–1129
10. Walker, L.M. and Burton, D.R. (2018) Passive immunotherapy of viral infections: 'super-antibodies' enter the fray. *Nat. Rev. Immunol.* 18, 297–308
11. Parry, H.A. et al. (2020) Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives. *Int. Immunopharmacol.* 85, 106639
12. Basalp, A. and Yucel, F. (2003) Development of mouse hybridomas by fusion of myeloma cells with lymphocytes derived from spleen, lymph node, and bone marrow. *Hybrid Hybridomics* 22, 329–331
13. de Almeida, R. et al. (2018) Enhanced immunization techniques to obtain highly specific monoclonal antibodies. *MAbs* 10, 46–54
14. Staszewski, R. (1984) Cloning by limiting dilution: an improved estimate that an interesting culture is monoclonal. *Yale J. Biol. Med.* 57, 865–868
15. Carter, P.J. (2006) Potent antibody therapeutics by design. *Nat. Rev. Immunol.* 6, 343–357
16. Zhang, C. (2012) Hybridoma technology for the generation of monoclonal antibodies. *Methods Mol. Biol.* 901, 117–135
17. Yu, X. et al. (2008) An optimized electrofusion-based protocol for generating virus-specific human monoclonal antibodies. *J. Immunol. Methods* 336, 142–151
18. Stevens, R.H. et al. (1979) Characterization of a circulating subpopulation of spontaneous antitetanus toxoid antibody producing B cells following *in vivo* booster immunization. *J. Immunol.* 122, 2498–2504
19. Morbach, H. et al. (2010) Reference values for B cell subpopulations from infancy to adulthood. *Clin. Exp. Immunol.* 162, 271–279
20. Greenfield, E.A. (2019) Preparing feeder cell cultures to support hybridoma growth. *Cold Spring Harb. Protoc.* Published online November 1, 2019. <https://doi.org/10.1101/pdb.prot103168>
21. Rems, L. et al. (2013) Cell electrofusion using nanosecond electric pulses. *Sci. Rep.* 3, 3382
22. Moraes, J.Z. et al. (2021) Hybridoma technology: is it still useful? *Curr. Res. Immunol.* 2, 32–40
23. Harris, J.F. et al. (1990) Spontaneous and radiation-induced genetic instability of heteromyeloma hybridoma cells. *Mol. Biol. Med.* 7, 485–493
24. Castillo, F.J. et al. (1994) Hybridoma stability. *Dev. Biol. Stand.* 83, 55–64
25. Kessler, N. et al. (1993) Stability of a murine hybridoma is dependent on the clonal line and culture media. *In Vitro Cell Dev. Biol.* 29A, 203–207
26. Chiorazzi, N. et al. (1982) Use of Epstein-Barr virus-transformed B cell lines for the generation of immunoglobulin-producing human B cell hybridomas. *J. Exp. Med.* 156, 930–935
27. Yu, X. et al. (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455, 532–536
28. Corti, D. and Lanzavecchia, A. (2014) Efficient methods to isolate human monoclonal antibodies from memory B cells and plasma cells. *Microbiol. Spectr.* 2. <https://doi.org/10.1128/microbiolspec.AID-0018-201>
29. Tickle, S. et al. (2015) A fully automated primary screening system for the discovery of therapeutic antibodies directly from B cells. *J. Biomol. Screen.* 20, 492–497
30. Layton, D. et al. (2013) Design and operation of an automated high-throughput monoclonal antibody facility. *Biophys. Rev.* 5, 47–55
31. De Masi, F. et al. (2005) High throughput production of mouse monoclonal antibodies using antigen microarrays. *Proteomics* 5, 4070–4081
32. Clargo, A.M. et al. (2014) The rapid generation of recombinant functional monoclonal antibodies from individual, antigen-specific bone marrow-derived plasma cells isolated using a novel fluorescence-based method. *MAbs* 6, 143–159
33. Walker, L.M. et al. (2009) Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* 326, 285–289
34. Macagno, A. et al. (2010) Isolation of human monoclonal antibodies that potently neutralize human cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128–131A complex. *J. Virol.* 84, 1005–1013
35. Corti, D. et al. (2013) Cross-neutralization of four paramyxoviruses by a human monoclonal antibody. *Nature* 501, 439–443
36. Traggiai, E. et al. (2004) An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. *Nat. Med.* 10, 871–875
37. Bonsignori, M. et al. (2017) Staged induction of HIV-1 glycan-dependent broadly neutralizing antibodies. *Sci. Transl. Med.* 9, eaai751419
38. Huang, J. et al. (2013) Isolation of human monoclonal antibodies from peripheral blood B cells. *Nat. Protoc.* 8, 1907–1915
39. Banchereau, J. et al. (1991) Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science* 251, 70–72
40. Good, K.L. et al. (2006) Kinetics of human B cell behavior and amplification of proliferative responses following stimulation with IL-21. *J. Immunol.* 177, 5236–5247
41. Kwakkenbos, M.J. et al. (2010) Generation of stable monoclonal antibody-producing B cell receptor-positive human memory B cells by genetic programming. *Nat. Med.* 16, 123–128
42. Casali, P. et al. (1986) Human monoclonals from antigen-specific selection of B lymphocytes and transformation by EBV. *Science* 234, 476–479
43. Kozbor, D. and Roder, J.C. (1981) Requirements for the establishment of high-titered human monoclonal antibodies against tetanus toxoid using the Epstein-Barr virus technique. *J. Immunol.* 127, 1275–1280
44. Steinitz, M. et al. (1977) EB virus-induced B lymphocyte cell lines producing specific antibody. *Nature* 269, 420–422
45. Krause, J.C. et al. (2011) A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. *J. Virol.* 85, 10905–10908
46. Corti, D. et al. (2010) Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. *PLoS One* 5, e8805
47. Bemasconi, N.L. et al. (2002) Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 298, 2199–2202
48. Nojima, T. et al. (2011) *In-vitro* derived germinal centre B cells differentially generate memory B or plasma cells *in vivo*. *Nat. Commun.* 2, 465
49. Haniuda, K. et al. (2017) *In vitro*-induced germinal center B cell culture system. *Methods Mol. Biol.* 1623, 125–133
50. Finney, J. and Kelsoe, G. (2021) Continuous culture of mouse primary B lymphocytes by forced expression of Bach2. *J. Immunol.* 207, 1478–1492
51. von Boehmer, L. et al. (2016) Sequencing and cloning of antigen-specific antibodies from mouse memory B cells. *Nat. Protoc.* 11, 1908–1923
52. Starkie, D.O. et al. (2016) Generation of recombinant monoclonal antibodies from immunised mice and rabbits via flow cytometry and sorting of antigen-specific IgG+ memory B cells. *PLoS One* 11, e0152282
53. Franz, B. et al. (2011) *Ex vivo* characterization and isolation of rare memory B cells with antigen tetramers. *Blood* 118, 348–357

54. Wang, Z. *et al.* (2020) Isolation of single HIV-1 Envelope specific B cells and antibody cloning from immunized rhesus macaques. *J. Immunol. Methods* 478, 112734
55. Smith, K. *et al.* (2009) Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat. Protoc.* 4, 372–384
56. Correa, I. *et al.* (2018) Evaluation of antigen-conjugated fluorescent beads to identify antigen-specific B cells. *Front. Immunol.* 9, 493
57. Setliff, I. *et al.* (2019) High-throughput mapping of B cell receptor sequences to antigen specificity. *Cell* 179, 1636–1646 e15
58. Woda, M. *et al.* (2016) Dynamics of dengue virus (DENV)-specific B cells in the response to DENV serotype 1 infections, using flow cytometry with labeled virions. *J. Infect. Dis.* 214, 1001–1009
59. Degaque, N. *et al.* (2013) Characterization of antigen-specific B cells using nominal antigen-coated flow-beads. *PLoS One* 8, e84273
60. Weitkamp, J.H. *et al.* (2003) Generation of recombinant human monoclonal antibodies to rotavirus from single antigen-specific B cells selected with fluorescent virus-like particles. *J. Immunol. Methods* 275, 223–237
61. Sanz, I. *et al.* (2019) Challenges and opportunities for consistent classification of human B cell and plasma cell populations. *Front. Immunol.* 10, 2458
62. Lin, Z. *et al.* (2014) *In vivo* antigen-driven plasmablast enrichment in combination with antigen-specific cell sorting to facilitate the isolation of rare monoclonal antibodies from human B cells. *Nat. Protoc.* 9, 1563–1577
63. Boonyaratnakornkit, J. and Taylor, J.J. (2019) Techniques to study antigen-specific B cell responses. *Front. Immunol.* 10, 1694
64. Gilman, M.S. *et al.* (2016) Rapid profiling of RSV antibody repertoires from the memory B cells of naturally infected adult donors. *Sci. Immunol.* 1, eaaj1879
65. Lei, L. *et al.* (2019) Antigen-specific single B cell sorting and monoclonal antibody cloning in guinea pigs. *Front. Microbiol.* 10, 672
66. Gross, A. *et al.* (2015) Technologies for single-cell isolation. *Int. J. Mol. Sci.* 16, 16897–16919
67. Efremova, M. *et al.* (2020) Immunology in the era of single-cell technologies. *Annu. Rev. Immunol.* 38, 727–757
68. Fitzgerald, V. and Leonard, P. (2017) Single cell screening approaches for antibody discovery. *Methods* 116, 34–42
69. Seah, Y.F.S. *et al.* (2018) Microfluidic single-cell technology in immunology and antibody screening. *Mol. Asp. Med.* 59, 47–61
70. Lin, W.N. *et al.* (2020) The role of single-cell technology in the study and control of infectious diseases. *Cells* 9, 1440
71. Nguyen, D.C. *et al.* (2018) Factors of the bone marrow microenvironment that support human plasma cell survival and immunoglobulin secretion. *Nat. Commun.* 9, 3698
72. Khodadadi, L. *et al.* (2019) The maintenance of memory plasma cells. *Front. Immunol.* 10, 721
73. Winters, A. *et al.* (2019) Rapid single B cell antibody discovery using nanopens and structured light. *MAbs* 11, 1025–1035
74. Jorgolli, M. *et al.* (2019) Nanoscale integration of single cell biologics discovery processes using optofluidic manipulation and monitoring. *Biotechnol. Bioeng.* 116, 2393–2411
75. Burman, L. *et al.* (2020) Isolation of monoclonal antibodies from anti-synthetase syndrome patients and affinity maturation by recombination of independent somatic variants. *MAbs* 12, 1836718
76. Love, J.C. *et al.* (2006) A microengraving method for rapid selection of single cells producing antigen-specific antibodies. *Nat. Biotechnol.* 24, 703–707
77. Jin, A. *et al.* (2009) A rapid and efficient single-cell manipulation method for screening antigen-specific antibody-secreting cells from human peripheral blood. *Nat. Med.* 15, 1088–1092
78. Ogunniyi, A.O. *et al.* (2009) Screening individual hybridomas by microengraving to discover monoclonal antibodies. *Nat. Protoc.* 4, 767–782
79. Kishi, H. *et al.* (2012) Screening of antigen-specific antibody-secreting cells. *Methods Mol. Biol.* 853, 141–150
80. Fitzgerald, V. *et al.* (2015) Exploiting highly ordered subnanoliter volume microcapillaries as microtools for the analysis of antibody producing cells. *Anal. Chem.* 87, 997–1003
81. Gerard, A. *et al.* (2020) High-throughput single-cell activity-based screening and sequencing of antibodies using droplet microfluidics. *Nat. Biotechnol.* 38, 715–721
82. Bounab, Y. *et al.* (2020) Dynamic single-cell phenotyping of immune cells using the microfluidic platform DropMap. *Nat. Protoc.* 15, 2920–2955
83. Mazutis, L. *et al.* (2013) Single-cell analysis and sorting using droplet-based microfluidics. *Nat. Protoc.* 8, 870–891
84. El Debs, B. *et al.* (2012) Functional single-cell hybridoma screening using droplet-based microfluidics. *Proc. Natl. Acad. Sci. U. S. A.* 109, 11570–11575
85. Shembekar, N. *et al.* (2018) Single-cell droplet microfluidic screening for antibodies specifically binding to target cells. *Cell Rep.* 22, 2206–2215
86. Ding, R. *et al.* (2020) Rapid isolation of antigen-specific B-cells using droplet microfluidics. *RSC Adv.* 10, 27006–27013
87. Brouzes, E. *et al.* (2009) Droplet microfluidic technology for single-cell high-throughput screening. *Proc. Natl. Acad. Sci. U. S. A.* 106, 14195–14200
88. Sista, R.S. *et al.* (2008) Heterogeneous immunoassays using magnetic beads on a digital microfluidic platform. *Lab Chip* 8, 2188–2196
89. Craig, F.F. (2018) The future of antibody discovery and cell line development. *Genet. Eng. Biotechnol. News* 38, 18–19
90. Lecault, V. *et al.* (2011) High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. *Nat. Methods* 8, 581–586
91. Rიცოვა, M. *et al.* (2013) Dissecting genealogy and cell cycle as sources of cell-to-cell variability in MAPK signaling using high-throughput lineage tracking. *Proc. Natl. Acad. Sci. U. S. A.* 110, 11403–11408
92. Gonzalez-Munoz, A.L. *et al.* (2016) Phenotypic screening: the future of antibody discovery. *Drug Discov. Today* 21, 150–156
93. Rajan, S. *et al.* (2018) Recombinant human B cell repertoires enable screening for rare, specific, and natively paired antibodies. *Commun. Biol.* 1, 5
94. DeKosky, B.J. *et al.* (2013) High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. *Nat. Biotechnol.* 31, 166–169
95. DeKosky, B.J. *et al.* (2015) In-depth determination and analysis of the human paired heavy- and light-chain antibody repertoire. *Nat. Med.* 21, 86–91
96. Sinha, N. *et al.* (2018) Integrating immunology and microfluidics for single immune cell analysis. *Front. Immunol.* 9, 2373
97. Wang, B. *et al.* (2018) Functional interrogation and mining of natively paired human VH:VL antibody repertoires. *Nat. Biotechnol.* 36, 152–155
98. Hoogenboom, H.R. (2002) Overview of antibody phage-display technology and its applications. *Methods Mol. Biol.* 178, 1–37
99. Parola, C. *et al.* (2019) Antibody discovery and engineering by enhanced CRISPR-Cas9 integration of variable gene cassette libraries in mammalian cells. *MAbs* 11, 1367–1380
100. Wang, B. *et al.* (2016) Discovery of high affinity anti-ricin antibodies by B cell receptor sequencing and by yeast display of combinatorial VH:VL libraries from immunized animals. *MAbs* 8, 1035–1044
101. Macosko, E.Z. *et al.* (2015) Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* 161, 1202–1214
102. Truck, J. *et al.* (2015) Identification of antigen-specific B cell receptor sequences using public repertoire analysis. *J. Immunol.* 194, 252–261
103. Wang, B. *et al.* (2015) Facile discovery of a diverse panel of anti-Ebola virus antibodies by immune repertoire mining. *Sci. Rep.* 5, 13926
104. Goldstein, L.D. *et al.* (2019) Massively parallel single-cell B-cell receptor sequencing enables rapid discovery of diverse antigen-reactive antibodies. *Commun. Biol.* 2, 304
105. Cao, Y. *et al.* (2020) Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell* 182, 73–84 e16
106. Horns, F. *et al.* (2020) Memory B cell activation, broad anti-influenza antibodies, and bystander activation revealed by single-cell transcriptomics. *Cell Rep.* 30, 905–913 e6

107. Croote, D. *et al.* (2018) High-affinity allergen-specific human antibodies cloned from single IgE B cell transcriptomes. *Science* 362, 1306–1309
108. Durham, N.D. *et al.* (2019) Broadly neutralizing human antibodies against dengue virus identified by single B cell transcriptomics. *eLife* 8, e52384
109. Mathew, N.R. *et al.* (2021) Single-cell BCR and transcriptome analysis after influenza infection reveals spatiotemporal dynamics of antigen-specific B cells. *Cell Rep.* 35, 109286
110. Michnick, S.W. and Sidhu, S.S. (2008) Submitting antibodies to binding arbitration. *Nat. Chem. Biol.* 4, 326–329
111. Carter, P.J. and Lazar, G.A. (2018) Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat. Rev. Drug Discov.* 17, 197–223
112. Jumper, J. *et al.* (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589
113. Graves, J. *et al.* (2020) A review of deep learning methods for antibodies. *Antibodies (Basel)* 9, 12
114. Laustsen, A.H. *et al.* (2021) Animal immunization, *in vitro* display technologies, and machine learning for antibody discovery. *Trends Biotechnol.* Published online March 25, 2021. <https://doi.org/10.1016/j.tibtech.2021.03.003>
115. Le Basle, Y. *et al.* (2020) Physicochemical stability of monoclonal antibodies: a review. *J. Pharm. Sci.* 109, 169–190
116. Spencer, S. *et al.* (2012) Solubility evaluation of murine hybridoma antibodies. *MAbs* 4, 319–325
117. Nixon, A.E. *et al.* (2014) Drugs derived from phage display: from candidate identification to clinical practice. *MAbs* 6, 73–85
118. Kaleli, N.E. *et al.* (2019) Phage display derived therapeutic antibodies have enriched aliphatic content: Insights for developability issues. *Proteins* 87, 607–618
119. Sun, A. and Benet, L.Z. (2020) Late-stage failures of monoclonal antibody drugs: a retrospective case study analysis. *Pharmacology* 105, 145–163
120. Garripelli, V.K. *et al.* (2021) Developability assessment for monoclonal antibody drug candidates: a case study. *Pharm. Dev. Technol.* 26, 11–20
121. Jefferis, R. (2016) Posttranslational modifications and the immunogenicity of biotherapeutics. *J Immunol Res* 2016, 5358272
122. Jenkins, N. *et al.* (2008) Post-translational modifications of recombinant proteins: significance for biopharmaceuticals. *Mol. Biotechnol.* 39, 113–118
123. Elhanati, Y. *et al.* (2015) Inferring processes underlying B-cell repertoire diversity. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 370, 20140243
124. Soto, C. *et al.* (2019) High frequency of shared clonotypes in human B cell receptor repertoires. *Nature* 566, 398–402
125. Rees, A.R. (2020) Understanding the human antibody repertoire. *MAbs* 12, 1729683
126. Lim, C.C. *et al.* (2019) Development of a phage display panning strategy utilizing crude antigens: isolation of MERS-CoV nucleoprotein human antibodies. *Sci. Rep.* 9, 6088
127. Sheets, M.D. *et al.* (1998) Efficient construction of a large non-immune phage antibody library: the production of high-affinity human single-chain antibodies to protein antigens. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6157–6162
128. Friedensohn, S. *et al.* (2018) Synthetic standards combined with error and bias correction improve the accuracy and quantitative resolution of antibody repertoire sequencing in human naïve and memory B cells. *Front. Immunol.* 9, 1401
129. IJspert, H. *et al.* (2015) Strategies for B-cell receptor repertoire analysis in primary immunodeficiencies: from severe combined immunodeficiency to common variable immunodeficiency. *Front. Immunol.* 6, 157
130. Chen, W.C. and Murawsky, C.M. (2018) Strategies for generating diverse antibody repertoires using transgenic animals expressing human antibodies. *Front. Immunol.* 9, 460
131. Dodd, R.B. *et al.* (2018) Therapeutic monoclonal antibodies to complex membrane protein targets: antigen generation and antibody discovery strategies. *BioDrugs* 32, 339–355
132. Chiarella, P. and Fazio, V.M. (2008) Mouse monoclonal antibodies in biological research: strategies for high-throughput production. *Biotechnol. Lett.* 30, 1303–1310
133. Aucouturier, J. *et al.* (2001) Adjuvants designed for veterinary and human vaccines. *Vaccine* 19, 2666–2672
134. Zaroff, S. and Tan, G. (2019) Hybridoma technology: the preferred method for monoclonal antibody generation for *in vivo* applications. *Biotechniques* 67, 90–92
135. Chen, D. *et al.* (2000) Epidermal immunization by a needle-free powder delivery technology: immunogenicity of influenza vaccine and protection in mice. *Nat. Med.* 6, 1187–1190
136. Chambers, R.S. and Johnston, S.A. (2003) High-level generation of polyclonal antibodies by genetic immunization. *Nat. Biotechnol.* 21, 1088–1092
137. Ning, Y. *et al.* (2006) An alternative strategy for high throughput generation and characterization of monoclonal antibodies against human plasma proteins using fractionated native proteins as immunogens. *Proteomics* 6, 438–448
138. Larsson, K. *et al.* (2006) Multiplexed PreST immunization for high-throughput affinity proteomics. *J. Immunol. Methods* 315, 110–120
139. Moody, M.A. and Haynes, B.F. (2008) Antigen-specific B cell detection reagents: use and quality control. *Cytometry A* 73, 1086–1092
140. Sanders, R.W. *et al.* (2013) A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS Pathog.* 9, e1003618