# 3 Naïve Human Antibody Libraries for Infectious Diseases

Soo Khim Chan, Anizah Rahumatullah, Jing Yi Lai, and Theam Soon Lim

**Abstract**

Many countries are facing an uphill battle in combating the spread of infectious diseases. The constant evolution of microorganisms magnifies the problem as it facilitates the re-emergence of old infectious diseases as well as promote the introduction of new and more deadly variants. Evidently, infectious diseases have contributed to an alarming rate of mortality worldwide making it a growing concern. Historically, antibodies have been used successfully to prevent and treat infectious diseases since the nineteenth century using antisera collected from immunized animals. The inherent ability of antibodies to trigger effector mechanisms aids the immune system to fight off pathogens that invades the host. Immune libraries have always been an important source of antibodies for infectious diseases due to the skewed repertoire generated post infection. Even so, the role and ability of naïve antibody libraries should not be underestimated. The naïve repertoire has its own unique advantages in generating antibodies against target antigens. This chapter will highlight the concept, advantages and application of human naïve libraries as a source to isolate antibodies against infectious disease target antigens.

**Keywords**

Naïve antibody library · Infectious diseases · Monoclonal antibodies · Phage display

## 3.1 Introduction

The two halves of the human immune system is divided as the innate and adaptive immune system, with the former being less specific as suppose to the latter. The innate immunity is the first line of defence against infections casting a wide protective net against foreign proteins. The work horse of the innate immune response is mostly present before the onset of infections and are not disease specific. The cellular and molecular components associated with the innate immune response like lysozyme, interferons, complement and toll-like receptors function by means of recognizing different classes of molecules unique to frequently encountered pathogens [1]. On the other hand, the adaptive immune system is highly

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T. S. Lim (ed.), *Recombinant Antibodies for Infectious Diseases*, Advances in Experimental Medicine and Biology 1053, https://doi.org/10.1007/978-3-319-72077-7_3

specific and is capable of recognizing specific foreign microorganisms and antigens to selectively eliminate them from the body. It differs from the innate immune response, as it is mainly a reaction towards a specific challenge. The adaptive immune response showcases four critical attributes that allows it to be effective in response to an infection. It shows high antigenic specificity, requires the generation of an elevated diversity of recognition entities, exhibits immunologic memory to allow a heighten response towards subsequent encounters of the same pathogen and permits recognition of self from non-self to elevate the risk of inappropriate response to self-components [2]. More importantly, one should note that the innate and adaptive immune responses actually functions in sync in a cooperative manner instigating a more efficient combined response than the individual response [1, 3–5].

The adaptive immune response has two major groups of cells, mainly antigen-presenting cells (APC) and lymphocytes. APC like macrophages and dendritic cells do not exhibit antigen-specific receptors but they function by processing and presenting the antigens to the antigen-specific receptors on T-cells. Lymphoytes are categorised further into two distinct cell types, namely the B-lymphocytes and T-lymphocytes [6]. B-lymphocytes are essential components that protect us against invasive antigens from the environment. The B-lymphocytes upon interaction with target antigens will proliferate and produce soluble forms of the B-cell receptors commonly known as antibodies [7]. The human immune system has the ability to produce a diverse collection of unique antibodies targeting a wide range of targets [8, 9]. These antibodies are circulating in the blood and lymphatic system to encounter foreign antigens [10]. The manner by which B-cell receptors are capable of demonstrating high target specificity is hypothesised using the clonal selection theory.

At the molecular level, B-cells undergo several complex stages of development to become fully activated antibody producing cells. However, the complex diverse nature of antibody development involves genetic rearrangement and somatic hypermutation, which is crucial for the immune system to fight off any possible foreign antigens encountered [11]. In this chapter, we will give a short overview of B-lymphocyte development including the repertoire generation processes. In addition to that, we will also highlight the concept and utilisation of the naïve B-lymphocyte repertoire in phage display library generation focusing on infectious diseases.

### 3.1.1 Diversification of B-Cell Repertoire

B lymphocytes, named after their discovery from bursa of Fabricius or bone marrow are differentiated from pluripotent hematopoietic stem cells [12]. Pre-B cells are generated from progenitor cells (pro-B cells) and migrate into the fetal liver during early embryonic development [13]. In the fetal liver, they develop and mature into B lymphocytes which mainly settles in epithelia, lung and gut-associated lymphoid tissues [14]. B-cell development at the early bone marrow-dependent stages is concentrated along the functional rearrangement process of the heavy chain (HC) and light chain (LC) gene segments independently. This rearrangement process is capable of fabricating an extended B-cell repertoire, which is responsible for expressing a diverse pool of antibodies with an estimated diversity above $10^{12}$.

In general, the generation of diverse antibodies by the B-cell can be classified to two stages comprising of pre- and post-antigen stimulation. The repertoire that exist at pre-antigen stimulation is categorised as the preimmune repertoire with an estimated $10^{12}$ unique antibody molecules found in the human body. This initial repertoire is sufficient to isolate antibodies against a wide range of antigens [2]. The diversity of the preimmune antibody repertoire is generated by the allelic diversity of the variable gene segments, V(D)J recombination, junctional diversification, pairing of both heavy and light chains, and, receptor editing [15].

The variable gene (V-gene) of the HC is a result of the rearrangement of three different gene segments, being the variable (V), diversity (D)

and joining (J) segments. However, the LC gene is formed by the combination of only two gene segments, the V and J gene segments. Each gene segment exist in multiple copies and the selection of different combination of the gene segments for construction of the V-gene is random. This gene rearrangement allows the formation of the initial diversity of the antibody repertoire [16]. Human heavy chain V regions have at least 56 functional V gene segments, 23 D gene segments, and 6 J gene segments [17].

V(D)J recombination is the primary somatic gene rearrangement of V, D, and J gene segments prior to antigen exposure [15, 18]. This mechanism happens in variable regions of HC and LC which encodes the antigen recognition sites. The sequence of formation allows the HC to be assembled prior to the LC [19]. V(D)J recombination is initiated by the introduction of DNA double-strand breaks using recombination activating gene (RAG) proteins (RAG 1 and RAG 2) at specific recombination signal sequences (RSS). The absence of these RAG proteins can lead to the failure of lymphocyte development at progenitor stages where V(D)J recombination occurs [20, 21]. The recombination process is guided by a set of conserved flanking DNA sequences located next to the gene segments to ensure correct recombination. These flanking DNA sequences are known as RSS which are conserved heptamer and nanomer sequences separated by a spacer which is either 12 or 23 bp in length [4, 19]. The joining of two gene segments during V(D)J recombination will adapt the 12/23 rule. The V and J gene segments are flanked by a pair of 23 bp spacers while D gene segments have 12 bp spacers flanking at both ends. Therefore, this allows the joining of D gene segments to both V and J gene segments but ensures that V gene segments will not combine with J gene segments directly [4]. Binding of RAG proteins to the RSS allows the formation of complexes to initiate a DNA double-strand break (DSB) in between the gene segments and RSS. The breaks are later joined by nonhomologous end-joining (NHEJ) proteins by the addition or deletion of nucleotides. This addition and deletion to the genes will further enhance the diversity of the variable regions by junctional diversification. However, not all genes produced after junctional diversification are functional, which will eventually lead to the death of these B cells [2]. The gene diversification process can also lead to the formation of B cell receptors that target self-antigens. This population of B cells that targets self-antigens will undergo a further editing process termed as receptor editing. Receptor editing functions to eliminate self-reactive B cells from the system. These B cells will be stimulated to undergo further V(D)J recombination in order to edit the receptors which further enhance the diversity of antibodies [18].

There is further antibody gene diversification that occurs post-antigen exposure with the ultimate aim to further diverse the repertoire. Upon exposure to antigens, antibodies perform a second wave of diversification to enhance the binding affinities against antigens via affinity maturation. Cumulative point mutations in both V regions of heavy and light chains induce affinity maturation of antibodies [2]. Somatic hypermutation (SHM) and class switch recombination (CSR) are the examples which lead to affinity maturation of the antibodies upon stimulation by antigens [22]. SHM diversifies the V regions and alter the affinity of antigen binding sites while CSR changes the heavy chain constant ($C_H$) region to generate different antibody isotypes (IgA, IgD, IgE, IgG, and IgM) [23, 24]. Both SHM and CSR are initiated by somatic mutations in V regions and constant regions respectively [25, 26]. It was also reported that activation-induced cytidine deaminase (AID) is required by both SHM and CSR to trigger the mechanisms [27, 28]. AID is a 24 kDa protein that deaminates cytidine residues on single-stranded DNA (ssDNA), not RNA or double stranded DNA (dsDNA) [23]. AID causes the formation of DNA lesions which is later repaired by multiple DNA repair pathways such as base excision repair (BER), mismatch repair (MMR), and NHEJ proteins [29]. The role of AID in the diversification process is evident as AID deficient mice and humans are unable to carry out SHM and CSR [30]. Despite happening at the same stage of B

cell differentiation, SHM and CSR are different biochemical processes which are mediated by different sets of enzymes [24]. SHM occurs in the germinal centers (GCs) while CSR can occur either inside or outside GCs [31–33].

SHM is a region specific mechanism which begins 150–200 base pairs (bp) downstream from the transcription start site (TSS) and is detected as far as 2 kb within the intronic region between J and C exons [34, 35]. AID deaminates cytosine residues in the V regions where the products of deamination lead to uracil-guanine (U-G) mismatch. This mismatch is later repaired by DNA repair mechanisms such as base excision repair and mismatch repair [36]. Incorporation of point mutations by SHM in V(D)J exons of both HC an LC helps to improve antibody affinities [37]. SHM occurs at a frequency of $10^{-5}$ to $10^{-3}$ mutations per base pair per generation, which is about a million times greater than the mutation rates in other genes [38]. Generally, single base substitution is preferable than insertions or deletions (indel) in SHM. Also, SHM has been reported to favour certain motifs such as WRCY (W = A or T, R = A or G, C, Y = T or C), and its reverse complement RGYW motifs [24, 39]. Therefore, SHM is active in CDR regions where these motifs are largely presented [40, 41]. About two third of SHM induced substitution mutations are transition mutations as they are more preferred in SHM [42]. Antibodies that have undergone SHM will produce higher affinity antigen receptors to compete with lower affinity or inactive antibodies. This selection process enables higher affinity antibodies against target specific antibodies to be concentrated [3].

CSR is a mechanism that allows the antibody isotypes to be switched to initiate different mechanisms at different sites in the body. CSR switches antibody isotypes via DNA deletion and recombination on the IgH constant region upon exposure to an antigen. Therefore, CSR is vital in antibody maturation against infections and vaccines. Defective CSR has been associated with a range of diseases [43–45]. CSR occurs between switch (S) sequences that are located upstream of each constant region genes ($C_H$) exon except for Cδ exons [46, 47]. Different immunoglobulin isotypes are encoded by different $C_H$ exons which are arranged in the order of Cμ, Cδ, Cγ, Cε and Cα in heavy chains [48]. Thereby, replacement of $C_H$ exons with Cγ, Cε and Cα could give rises to IgG, IgA or IgE respectively. However, the V regions of antibodies will remain intact and unchanged [32, 49]. IgD is the only immunoglobulin isotype of the five that is generated via alternative splicing of the primary transcripts that encode IgM but not via CSR [48].

The diversity generated via these molecular mechanisms is predominantly responsible for the assorted repertoire of the antibody genes. This variation provides the flexibility of the antibody repertoire to generate antibodies against a wide array of antigens with varying specificities and affinities. As the antibody repertoire is a reflection of the threats encountered by the immune system, different repertoires are formed as a result of that. Therefore variations in antibody repertoire in individuals of similar infections and different infections are expected. This will have a direct influence on the repertoire of antibodies that can be isolated from different antibody libraries used for phage display.

### 3.1.2 Antibody Phage Display Technology

Historically, the application of antibodies for biomedical applications was first shown with the application of antisera from hyperimmunized animals to treat botulism and diphtheria [50]. The use of antisera highlighted the potential of antibodies although in a polyclonal level as a magic bullet to target infections for treatment. It was not until the introduction of hybridoma technology that production of monoclonal antibodies (mAbs) was realised. Hybridoma technology requires the fusion of immortal myeloma cells with antibody producing spleen cells to generate a hybridoma exhibiting characteristics of both myeloma and spleen cells. This characteristic allows for the infinite generation of mAbs *in vitro* [51]. However, the approach requires the use of murine derived spleen cells post-immunization which have been reported to trigger human anti-mouse

antibody (HAMA) response upon administration. This greatly limits the therapeutic applications of murine mAbs in humans which may result in reduced efficacy over time and in some cases anaphylaxis [52]. The complications associated with the use of animal derived mAbs resulted in a string of technological advancements like CDR grafting and antibody humanization to reduce the "murine-nature" of the antibodies generated. Ultimately, the ability to generate fully human antibodies was perceived as the solution for this issue.

The introduction of phage display technology revolutionized the way antibodies was to be produced *in vitro* in the twentieth century. The application of *in vitro* display technologies like phage display has allowed the generation of fully human mAbs in greater amounts and in a more controlled manner [53, 59–61]. However, phage display is no longer the only display method available for the production of human mAbs. Examples of other *in vitro* display technologies that can be used for mAb generation are ribosomal display [54], yeast surface display [55, 56], bacterial surface display [57, 58] and mRNA display [62, 63]. The introduction of the xenograph mice technology for human antibody generation also assisted in the generation of monoclonal antibodies with the prospect of affinity maturation [64]. Phage display is a promising *in vitro* display method that has been utilised successfully to isolate target specific antibodies by exploiting the Ff phages or filamentous phages [65]. The important advantages of phage display that makes it a preferred alternative is the robustness, simplicity, and stability of phage particles which allows the selection of desired antibodies against different targets under predefined conditions [66].

Phage display technology was first introduced by George Smith in 1985 to display peptides on the surface of phage particles as a fusion to the coat proteins [67] which was ultimately exchanged with antibody fragments for the isolation of mAbs [68]. Due to the non-lytic characteristic of filamentous phages (f1, fd, M13), they are commonly used in phage display to infect gram negative bacteria carrying the F pilus [69]. The fundamental concept of phage display lies in the direct physical linkage between genotype and phenotype [70]. The proteins or peptides encoded by the genotype are usually displayed on the phage surface by fusion with the phage coat proteins pVIII (p8) and pIII (p3) [71, 72] even though fusion to coat protein pVII [73, 74] and pIX [74, 75] have also been reported. The N-terminal of p3 was found to be vital for phage infectivity [76]. Hence, the protein of interest fused to p3 affects the efficiency of phage infectivity and propagation [77]. Phage display takes advantage of the natural process of phage infection and propagation by replicating the process with the aid of a helper phage system when using a phagemid vector. The helper phage is used in combination with the phagemid system to provide the necessary coat proteins as well as wild-type p3 for phage packaging as shown in Fig. 3.1. The phagemid vector is unique in that it is designed to contain the features from both a bacterial expression plasmid as well as a phage replication plasmid. The phagemid is normally designed to harbour the antibody gene as a fusion to gIII. Therefore the helper phage will function as the source of the remaining wild-type proteins required for phage replication, morphogenesis and assembly [78].

The process for mAb generation by antibody phage display requires the presence of antibody libraries harbouring approximately $10^9$ to $10^{11}$ phage particles, each presenting an unique antibody clone [79]. The physical isolation and enrichment of target specific mAbs from the diverse library is performed via biopanning. Biopanning is a term coined to describe the sieving process of positive clones from the diverse library and subsequent augmentation of the positive population. The biopanning process involves several repetitive cycles of binding, washing, and amplification of the positive phage clones until a predominant population is present [70]. The process involves the immobilization of the target antigen on various solid surfaces such as polystyrene plates, microbeads [80], nitrocellulose blots [81], column matrix [81] or immunoassay tips [59]. The antibody phage library is then introduced to the bound target antigen and left to incu-

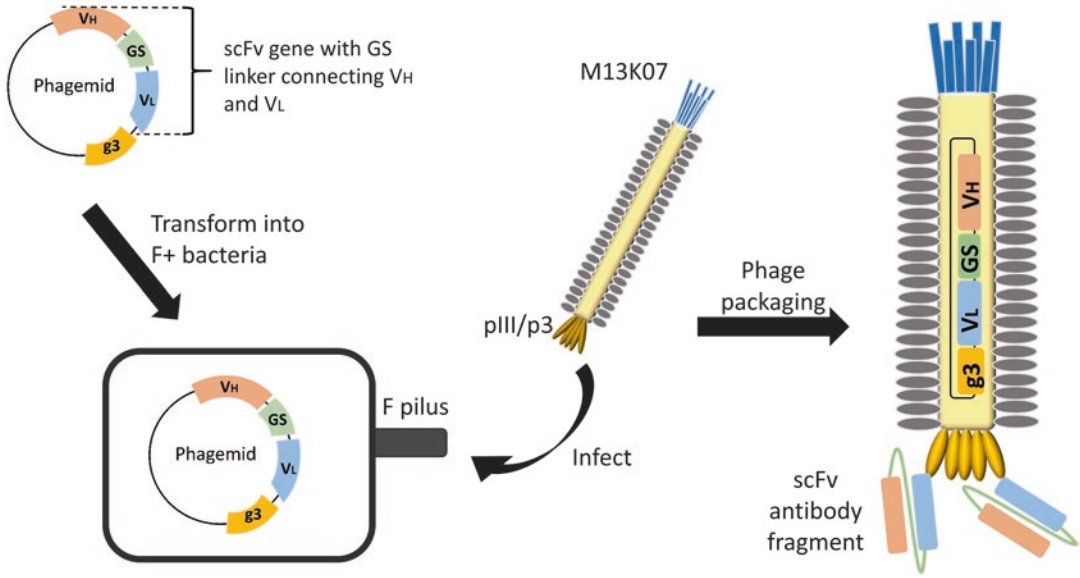


**Fig. 3.1** Schematic representation of phage particles bearing antibody fragments for display. The gene encoding the scFv fragment is fused to gIII in a phagemid before introduction into a bacteria carrying the F pilus. Infection of scFv fused gIII phagemid with a helper phage (M13K07) allows the helper phage to provide all necessary proteins required for phage packaging

bate to allow affinity based capture of the antibody bearing phage particles to the antigens. The unbound antibody-phage particles are then removed by washing. The remaining bound phage particles are then eluted using acid-based elution or enzyme based digestion. The eluted phage particles are then amplified by re-infection with *E. coli* for a fresh cycle of phage packaging. The new population of phage particles are then ready to be used for the ensuing round of biopanning. This cycle is repeated a few rounds to ensure a continuous enrichment of the targeted population of phage particles. Varying the wash and elution conditions can customize the characteristics of the isolated mAbs. This is to simulate different environments and stringencies to isolate mAbs featuring a particular characteristic.

The major limitation associated with the *E. coli* folding machinery is its ability to provide better display efficiencies of smaller versions of the antibody fragments instead of the full antibody format on phage surfaces [82]. However, the presentation of full length IgG using bacterial periplasmic display was previously demonstrated to yield antibodies with nanomolar range of binding affinities [83]. Even so, the use of smaller antibody fragments has a distinct advantage in terms of tissue penetration. Smaller antibody fragments are able to penetrate tissues with a higher efficacy without compromising their affinities and specificities. The diminutive size of antibody fragments that works in favour for bacterial expression host allows for an easier and faster expression of recombinant antibodies [84, 85]. Several common antibody formats displayed on phage includes the single-chain fragment variable (scFv) [86, 87] fragment antigen binding (Fab) [88, 89] and domain antibodies [85]. The scFv format is the preferred antibody format used for phage display as it is not prone to degradation and is easier to be expressed in its functional form by bacteria [90, 91]. The scFv format is made up of the variable heavy chain ($V_H$) and variable light chain ($V_L$) domains interconnected with a glycine-serine (GS) linker [70]. The application of linkers has also provided new Fab molecules in the form of single chain Fab (scFab) fragments for phage display [88]. However, the choice of fragment to be used for library generation will be dependent on the downstream appli-

cation of the antibodies. Possibilities to engineer new formats allow grafting of the variable domains from any format to be engineered to other subsequent formats. This freedom for format exchange allows for improved applications of antibodies for biomedical applications. Even so, the choice of format used will also have to take into account the ability to clone a large diverse collection of the clones to form a library for phage display selections. In addition to that, format conversions from scFv to full IgG have been reported to cause a loss of target affinity largely due to conformational changes in the structure post-conversion. Therefore, proper consideration and design is required before format exchange of antibody fragments is carried out [92].

## 3.2 Construction of Naïve Antibody Library for Phage Display

As highlighted earlier, the basic requirement for antibody phage display is the accessibility of an antibody library for screening. Antibody libraries are useful assets for antibody development programs as they can be applied for various targets for multiple applications. In general, antibody libraries are classified into four categories, namely the naïve, immune, semi-synthetic and synthetic libraries. The classification of the antibody libraries are mainly influenced by the source of antibody V genes for library generation [93]. Naïve antibody libraries are constructed using V genes from non-immunized donors [93, 94]. Immune antibody libraries however are constructed utilizing antibody V genes from immunized donors or infected individuals [93]. Synthetic and semi-synthetic libraries are constructed using synthesized DNA oligonucleotides where the repertoire diversity is devoid of any natural immune maturation processes [95]. This chapter will focus on the principles and characteristics of naïve libraries including the application of antibodies derived from naïve libraries in combating infectious diseases.

Naïve antibody libraries are constructed using B cells of unimmunized or healthy donors, normally focusing on the IgM isotype. Naïve libraries are supposedly able to be used for the isolation of mAbs against any antigen [96]. The process involved in the generation of naïve antibody libraries is well established and has been described in numerous publications [97–100]. The human natural antibody repertoire is approximately $10^{11}$ in size, which is a thousand times larger than the murine naïve antibody repertoire [101]. However, antibodies isolated from naïve antibody libraries usually exhibit lower affinities compared to antibodies isolated from immunized libraries. This is because the naïve repertoire would not have undergone *in vivo* affinity maturation to produce higher affinity antibodies [96]. During antibody library generation, several critical aspects that are monitored includes library size and repertoire diversity in determining the quality of the library [102]. In the case of naïve libraries, larger library sizes are preferred to ensure higher affinity mAbs are isolated. The correlation between larger library sizes with higher affinity mAbs has been reported in several instances as high affinity clones were successfully retrieved from larger size libraries compared to smaller size antibody libraries [93, 103, 104].

The actual source where antibody genes are retrieved from is a vital consideration during the construction process of antibody libraries. Antibody genes could be sourced from peripheral blood mononuclear cells (PBMC), bone marrow, tonsil, and, spleen [105]. However, B cells at various stages have different degrees of mutation. B cells obtained from the bone marrow are the most naïve while B cells from tonsils are the most mutated. The degree of mutation will have an adverse effect on the repertoire of the library. Therefore, the highly mutated V genes from tonsils is not an ideal choice for naïve antibody library construction but suitable for immune libraries [106]. Naïve B cells are better represented by the raw V(D)J recombination in the bone marrow which have yet to encounter any antigens. The diversity of a naïve antibody library

is largely dependent on the number of unique antibody sequences that is successfully cloned into the library [61]. B cells are usually taken from peripheral blood for the construction of human antibody libraries as the method for extraction is less invasive compared to bone marrow extraction [107]. Peripheral blood contains a high proportion of naïve B cells, making peripheral blood a very good source for B cells [61]. The first naïve antibody library reported was constructed using peripheral blood lymphocytes (PBLs) of unimmunized donors with an estimated library size of greater than $10^7$. The library was successfully used to isolate soluble antibody fragments against haptens with good affinities [108].

The general concern associated with naïve antibody libraries is the true nature of the sample naivety. This is due to the subjective nature of the term healthy, as it is unlikely that donors have never been infected throughout their lifetime making it realistically impossible to obtain "truly naïve" samples [94]. This is because healthy individuals would have recovered from a prior infection or have been immunized at some stage in their life. Vaccinations have been reported to increase antigen-specific repertoires which could result in a skewed repertoire being generated [109, 110]. In addition, memory cells in the immune system are capable of recognizing past infections which could contribute to changes in the antibody repertoire [94, 111]. The common considerations for naïve library sample collection includes healthy donors without immunosuppressive treatments as well as antigen exposure history [112], age [113, 114], genotype and chromatin structure [115, 116].

The general protocol for the construction of naïve antibody libraries using PBMC is demonstrated in Fig. 3.2. Naive antibody library construction starts with blood sample collection from a healthy population. B cells from whole blood can be isolated using the Ficoll-Hypaque density gradient centrifugation by utilizing the density differences between mononuclear cells

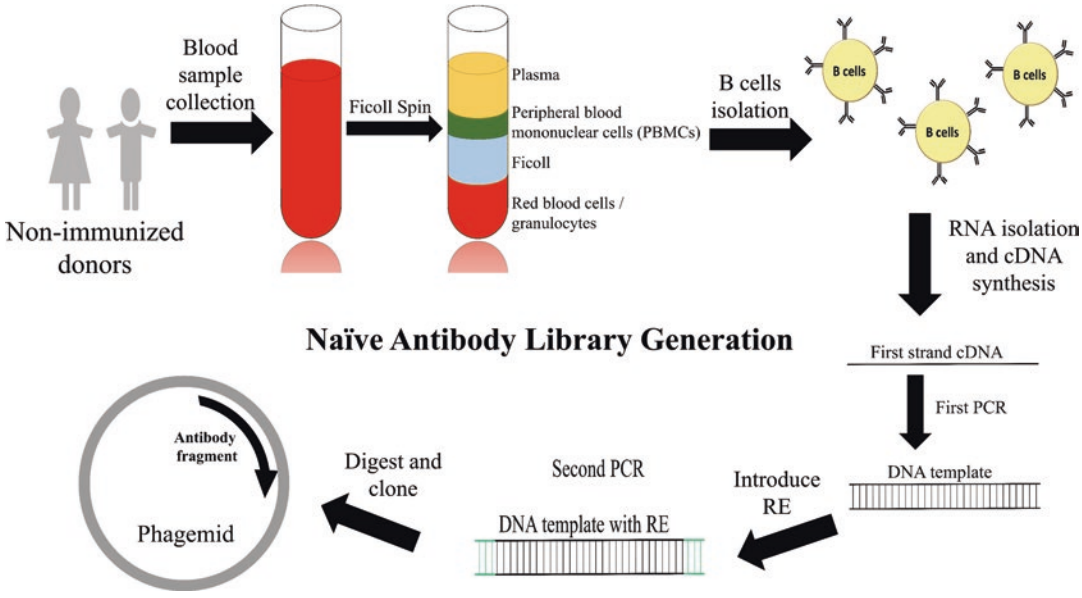


**Fig. 3.2** General steps involved in naïve antibody library generation. Generation of naïve antibody library starts with blood sample collection from healthy donors. Total RNA is then prepared and used as template for reverse transcription into first strand cDNA by using gene specific primers. The cDNA is then amplified by polymerase chain reaction (PCR) to generate DNA amplicons. Second round of PCR is performed on these DNA samples to introduce cut sites for restriction enzymes. The amplicons are then cloned into a phagemid and transformed into suitable bacteria cells

with other elements in the blood for separation [117]. Mononuclear cells such as lymphocytes, monocytes, and platelets stay on top of the Ficoll layer because they are not dense enough to penetrate the Ficoll layer. Granulocytes on the other hand sediment to the bottom of the Ficoll layer due to increased density upon contact with the Ficoll medium. Red blood cells also sediment to the bottom of Ficoll solution due to aggregation by Ficoll. Mononuclear cell layer is washed and centrifuged to remove platelets that remain in the supernatant [117].

Upon obtaining B cells, mRNA is isolated immediately and reversed transcribed into cDNA. Reverse transcription of mRNA can be carried out using either random hexamer primers or antibody specific primers. Antibody specific primers such as IgM constant region specific primers allow the cDNA synthesis of antibody sequences from the IgM isotype. However, priming using random hexamers allows all the five antibody classes (IgA, IgD, IgE, IgG, IgM) to be amplified for a highly diverse repertoire. Therefore, random hexamers are being used more often to synthesize first strand cDNA from B cell mRNA for the generation of naïve antibody libraries [105]. In most cases, the preferred isotype for naïve antibody library generation is IgM. However, up to 40% of the circulating memory cells expresses IgM that may not be naïve due to somatic hypermutation upon exposure to antigens [61].

Upon obtaining B cell cDNA, variable (V) regions of the heavy chain ($V_H$) and light chain ($V_L$) are amplified using a defined set of primers that encompasses the human antibody gene repertoires. Different primer sets have been published over the year for the amplification of all V genes [118, 119]. However, V gene primer designs have evolved over the years due to additional information available with improvements in sequencing technology and bioinformatics analysis. An improved database of antibody V gene sequences from next generation sequencing has shed much light into antibody gene usage and V gene family coverage. The addition of new antibody gene sequences has allowed for new primer sets to be designed with improved gene coverage [67]. Improved gene coverage of the primer set has a great influence on the repertoire of the final antibody library generated. This helps to ensure all possible antibody genes are represented in the library as well as increasing the possible combinations of the HC and LC genes.

The first V gene amplification is usually done with gene specific primers without the introduction of any flanking regions. Amplification bias during PCR is a common concern that could influence the final repertoire of the library. Antibody gene usage is not evenly distributed with variations resulting from previous infections with additional variations from individual immune responses. Therefore having a highly representative primer set is important to ensure that unique gene families or poorly presented templates are also amplified [61]. A second round of PCR amplification is performed to introduce the desired restriction sites for cloning into the phagemid vector. There are several different strategies to construct a naïve antibody library. The most common approaches used for the construction of naïve antibody libraries is either by two-step/three-step cloning [94, 120] or PCR assembly [108] as shown in Fig. 3.3 In two-step cloning, light chain genes are first amplified to introduce the glycine-serine linker before cloning into the phagemid vector to establish an intermediate library. Cloning of the amplified heavy chain genes to the intermediate library is done to form a full scFv format library. The orientation of which V gene to clone first is influenced by the higher diversity of the HC CDR3 regions [121]. In some cases, three-step cloning is used to construct naïve antibody libraries. In this approach, independent $V_H$ and $V_L$ libraries are first cloned to function as intermediate libraries. Then the libraries are digested and ligated together to form the full antibody library containing both $V_H$ and $V_L$ repertoires [102]. In PCR assembly, the $V_H$ and $V_L$ genes are amplified to introduce the GS linker before cloning. Then, PCR assembly is used to assemble the three fragments to form the full scFv format. The assembled fragment is then digested with restriction enzymes before cloning it upstream of the gene III coat protein in the phagemid [97].

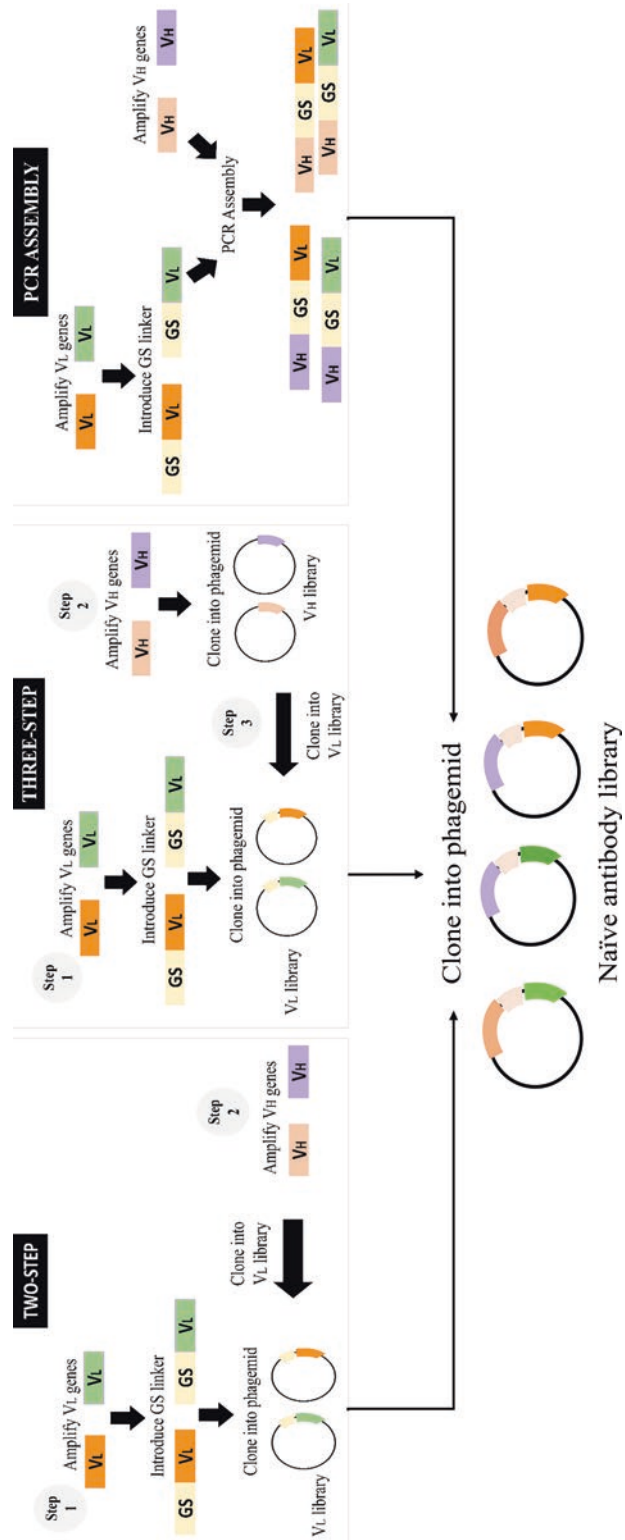


**Fig. 3.3** Possible approaches for naïve library generation

Ligated products of antibody sequences and phagemids are transformed into bacterial cells carrying the F pilus such as XL1-Blue MRF'[97], ER2738 [122], TOP10F'[123], *E. coli* SS 320-M13cp [124, 125] and TG1 [120, 126]. The choice of cells that are used must have high transformation efficiencies. Even so, a high number of transformation is required to overcome the limitation of *E. coli* transformation in order to generate large naïve antibody libraries [127]. The library diversity is normally calculated based on the number of colonies formed from serial dilutions of transformants that are plated out on 2xYT agar and incubated overnight at 37 °C [97, 128]. Library developers often target for the highest library size as possible. However, this is an uphill task due to the limitations mentioned earlier. Even so, the average size for naïve antibody libraries is in the range of $10^9$ or higher. The direct correlation between library size with antibody affinity has spurred the need to generate large library sizes in order to isolate high affinity antibodies without the need to further improve the affinities [61].

## 3.3 Isolation of Naïve Antibodies from Human Antibody Library Against Infectious Diseases

There is a growing concern in terms of global health with the emergence of new infectious diseases and the re-emergence of old diseases. This situation is intensifying concerns that these diseases might eventually become pandemics [129, 130]. The advent of antibody based technologies has a direct impact on global health as antibodies are useful biomolecules for diagnostics and therapeutic applications. The application of antibodies in diagnostics allows for a major role in disease management and early detection systems. This is critical in the fight against infections and disease prevention. In the last decade, the identification of antibiotic resistance has highlighted the vulnerability of the human population towards new infections. This highlighted the need for new alternative treatments which includes antibody based therapies [131]. Antibody based therapies appear to be a promising solution for infectious diseases due to its high specificity and the flexibility it provides in terms of modifications allows for direct programming of specific mechanisms for the treatment of specific diseases [132].

Generally, immune libraries are preferred for the generation of antibodies against infectious disease related targets due to the skewed antibody repertoires as a result of exposure to the infection [133]. In addition to that, high affinity antibodies could be isolated from immune libraries due to *in vivo* affinity maturation from antigen presentation [96]. Therefore, the preferential repertoire against a particular disease means that libraries of smaller sized repertoires are sufficient. One major drawback for immune libraries is the inability for it to be applied across a wide range of antigens. This means that new libraries have to be constructed each time antibodies against antigens from different infections have to be generated. The major advantage of naïve libraries is the huge diversity that does not show bias towards any antigen making it a universal source of antibodies against any target antigen [96, 133].

### 3.3.1 Naïve Library Derived Antibodies Against Target Antigens of Infectious Agents

The use of antibody based therapies started as early as the 1890 when sera from immunised animals was applied to cure bacterial infections with *Clostridium tetani* and *Corynebacterium diphtheriae* [134]. Since then, it has also been applied for other diseases such as tetanus, hepatitis A and B, measles, rabies, varicella, and vaccinia [135]. Over the years, the developments in antibody technology allowed for antibodies to be produced at a faster rate *in vitro*. This contributed to the increase of antibody discovery projects against many different diseases. Table 3.1 highlights the application of naïve human antibody libraries against infectious diseases caused by virus, bacteria, fungal and parasites.

**Table 3.1** Monoclonal antibodies against a list of infectious agents

| Infectious agent | Target | Antibody format | References |
|---|---|---|---|
| **Bacterial** | | | |
| *Bacillus subtilis* IFO 3336 | Native spores | scFv. Fab | Zhou et al. [139] |
| *Lactobacillus acidophilus* ATCC 4356 | Intact bacteria | scFv | Close et al. [143] |
| *Salmonella typhi* | Hemolysin E antigen | scFv | Lim et al. [94] |
| *Bacillus anthracis* | Protective antigen (PA83) | scFv | Cirino et al. [140] |
| *Mycobacterium tuberculosis* | 85 B | scFv | Fuchs et al. [141] |
| **Virus** | | | |
| H5N1 influenza virus | Hemagglutinin (HA) | Fab | Lim et al. [145] |
| Venezuelan equine encephalitis virus (VEEV) | Intact virus particles | scFv | Kirsch et al. [162] |
| Dengue | Nonstructural (NS) protein 5 | Fab | Zhao et al. [152] |
| Dengue | Nonstructural (NS) protein 3 | Fab | Moreland et al. [151] |
| Rabies virus | | scFv | Pansri et al. [156] |
| Hendra virus | Envelope G glycoprotein | Fab | Zhu et al. [157] |
| **Fungal** | | | |
| *Candida albicans* 3153A | Heavy and light chain variable regions | scFv | Haidaris et al. [160] |
| Fungal | Aflatoxin-BSA | scFv | Moghaddam et al. [163] |
| *Aspergillus fumigatus* | Glycosylhydrolase Crf2 | scFv | Schütte et al. [164] |
| **Parasite** | | | |
| *Plasmodium falciparum* | Histidine rich protein 2 (rPfHRP2) | scFv | Leow et al. [165] |

Antibody generation using traditional methods that require a living host is difficult when dealing with targets that are toxic to the host. This can be overcome by using naïve antibody libraries coupled with antibody phage display technology to allow the isolation of antibodies against antigens which are toxic or detrimental to the host [79]. For instance, hemolysin E (HlyE) toxin produced by *Salmonella enterica serovar* Typhi is a pore forming toxin which is antigenic to humans, may pose a challenge for conventional antibody generation techniques [136]. However, soluble scFv mAb against HlyE toxin was successfully isolated from a $2 \times 10^9$ naïve human scFv library constructed from 90 healthy donors. The donors consisted of equal distribution of donors from three ethnic groups (Malay, Chinese, and Indian) and equal gender distribution. To enhance the diversity of the library, combinatorial mixing of $V_H$ and $V_L$ chains were performed where heavy and light chains were randomly mixed. The naïve library was generated by two-step cloning by inserting the light chains followed by heavy chains [94]. The ability to harness the contrasting immune responses from different individuals resulting in varying gene rearrangements, gene pairings and usage allows for the improved diversity of the naïve library. This improved diversity ensures higher repertoire for antibody generation with improved affinities. The large library size also has a significant role in determining the antibody quality isolated from the library.

Another primary example of the application of naïve libraries is the ability to generate antibodies against the spores of *Bacillus anthracis* which can result in death post inhalation. The spores of *B. anthracis* is a potent biological threat which was abused as a bioweapon in 2001 when anthrax spores were intentionally mailed to Washington, DC [137, 138]. The toxic nature of the spores makes immunization for antibody generation

impossible as it would most likely kill the host. Therefore, isolation of human antibodies capable of binding to live native spores is possible using a naïve scFv library. Biopanning of a naïve scFv library isolated two clones with differences in specificity and affinity to the spores of the Bacillus strains as well as recognizing different epitopes. Analysis of both clones revealed a bias in $V_H$-$V_L$ pairing in the repertoire after exposure of the spores to the human immune system. A naïve Fab library was later constructed by combining $V_H$ and $V_L$ genes from the isolated positive scFv clones with the aim to reduce the cross reactivity with other Bascillus strains. Chain shuffling technique was introduced to the Fab library to increase the diversity of $V_H$ and $V_L$ genes. Clones isolated from Fab library showed much lower cross reactivity with spores from other strains [139]. The process was envisioned from the panning of the human naïve scFv library against the spores of *Bacillus subtilis* IFO 3336 [139].

Another published work on *Bacillus anthracis* was focus at the protective antigen (PA83) which is essential for the anthrax toxicity mechanism. Several scFvs binding to PA83 were isolated from a naïve library where the highest binder exhibited $K_d$ of 50 nM. The library was generated via three-step cloning where both the $V_H$ and $V_L$ genes were first cloned in separate plasmid vectors to generate separate $V_H$ and $V_L$ libraries. The advantage of individual libraries is to supply endless material for scFv assembly [108]. The size of naïve scFv library constructed was $6.7 \times 10^9$ [140].

A naïve human antibody library has also been used to isolate antibody fragments against tuberculosis (TB) antigens [141]. TB appears to be one of the leading fatal microbial infectious diseases worldwide caused by *Mycobacterium tuberculosis* [142]. A naïve library constructed using B cells from 44 donors of Caucasian, African, Indian and Chinese origin was used. Two-step cloning was applied to clone both the heavy and light chains of antibodies to create two libraries with the same heavy chain repertoire but differ in the light chain (lambda and kappa) repertoire. The libraries were validated with 110 antigens to ensure the quality of the libraries [121]. The libraries were used to pan against the antigenic 85 B complex of Mtb. Five scFv clones were successfully isolated and confirmed with DNA sequencing. Four clones have lambda light chain and one with kappa light chain [141].

The ability of phage display methodology to be applied to an array of different target types allows for the isolation of antibodies against various targets. A naïve antibody library was also used to pan against intact bacteria for antibody generation. The naïve scFv library isolated species-specific antibodies against *Lactobacillus acidophilus* ATCC 4356A prior to FACS quantification and genome sequencing. Generation of this naïve scFv library incorporated the use of cre-lox system which is able to generate a highly diverse library with the size of $3 \times 10^{11}$. The phagemid with the lox site was used to infect Cre recombinase expressing bacteria, where a high number of phagemids were able to enter a single bacterium where shuffling between $V_H$ and $V_L$ occurred. The lox site was introduced between $V_H$ and $V_L$ genes to act as the scFv linker. Selection of lox site was restricted to a single basic amino acid to reduce proteolysis, removal of stop codons and is least hydrophobic in nature. Analysis showed the isolated scFv recognised the surface layer A (SlpA) protein of *L. acidophilus* which is abundant and the antibody demonstrated high degree of specificity [143].

Hemagglutinin (HA) regions of influenza viruses are responsible for antigenicity of influenza viruses. Extensive mutation of these regions enabled the emergence of multiple antigenically distinct H5N1 strains [144]. To overcome this issue, targeting conserved regions of protein outside the antigenic sites becomes one of the option to avoid virus escape. A combination of panning strategies was performed to isolate Fab-phage antibodies away from HA antigenic sites, towards the conserved regions of HA. Thirty three Fab antibody fragments against multiple HA were successfully isolated from naïve human Fab phage display library HX01 (Humanyx Pte Ltd., Singapore). Five out of these 33 antibodies exhibited specificity towards the conserved region HA2 which is vital for fusion of viral and cell membrane [145].

Aside from isolating antibodies against the intact bacteria, naïve antibody phage library was also applied successfully to isolate antibodies against intact virus particles. Human naïve scFv library HAL4/7 was applied to pan against intact virus particles of Venezuelan equine encephalitis virus (VEEV). This is very interesting as most publications have shown isolation of naïve antibodies against virus proteins and not the intact virus particles [146–148]. The size of the naïve scFv library applied is $5 \times 10^9$ and was subjected to three rounds of panning. Preselection step was performed by incubating the phage library with concentrated supernatant of non-infected Vero cells. To minimize false positive results, soluble scFvs were used in ELISA instead of scFv phage. The 11 isolated scFvs were analysed by integrative database of germ-line variable genes from immunoglobulin loci of human (VBASE2). Results demonstrated the variable domains of light chains consisting of 1, 2, 3, and 6 while 1, 3, and 4 for heavy chains.

Dengue virus of the *Flaviviridae* family has a single stranded positive strand RNA as the genome. Post translational modification of the polypeptide give rise to three structural proteins (capsid, membrane, envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [149, 150]. Non-structural 3 (NS3) protein and non-structural 5 (NS5) proteins are essential in viral replication which are subdivided into few distinct functional domains. Targeting these domains provides important information about dengue replication and for the development of inhibitors against dengue virus [151, 152]. Naïve human Fab library HX02 (Humanyx Pte Ltd., Singapore) was used to screen for NS3 binders. NS3 proteins were biotinylated to bind to streptavidin magnetic beads prior to panning. Concentration of immobilised NS3 proteins was reduced for each round with an increase in the washing steps during panning. Ten unique clones were obtained and observed to bind to the protease domain, helicase domain, or both the domains for different dengue serotypes. One clone 3F8 demonstrate the ability to bind with NS3 proteins from all dengue serotypes (DENV1-4) and has a high binding affinity (15 nM) in ELISA as well as surface plasmon resonance (SPR) with $K_D$ 0.5 nM [151]. Similar panning strategy was applied to isolate antibodies against the NS5 proteins. However, the six unique clones isolated were specific only to dengue virus serotype 3. Hence, panning was performed with alternate dengue serotypes to enrich clones which were able to recognise NS5 proteins for all serotypes. Two cross-reactive Fabs (5M1 and 5R3) and one DENV3 specific Fab (5M3) were obtained. The clones were incubated with an array of overlapping 15-mer peptides spanning the domain regions to map the epitope binding sites [152]. The ability to isolate cross-reactive clones against several serotypes as well as serotype specific antigens shows the flexibility afforded when using naïve antibody libraries.

Various endeavours have been taken to select good binders from antibody phage libraries against various antigens. Approaches such as increased library sizes [96, 100], enhanced cloning strategies [153], and improve panning protocols [154] have been demonstrated to retrieve good binders. Another approach reported the use of protease sensitive helper phage KM13. This method allows high throughput screening of naive library for a wide range of antigens [155, 156]. The protease-cleavage sequence was inserted between domain 2 and domain 3 of pIII to eliminate background contributed by helper phage. With this KM13 helper phage, one or two rounds of selection would be needed since the number of selection did not increase the chance to obtain new binders. Naïve scFv against rabies virus was successfully isolated from a human naïve scFv library with the size of $1.5 \times 10^8$ individual clones. Rabies virus is considered a difficult target due to the presence of limited amount of target in the mixture which might contribute to the enrichment of background or non-specific binders [156].

*Hendra* virus (HeV) belongs to the *Henipavirus* genus of the *Paramyxovirinae* which depends on two major membrane-anchored envelope glycoproteins (G and F) for infection. This virus can cause mortality in both animal and human hosts by infecting cells with the envelope

glycoproteins fused to cell membranes. Naïve Fab antibody library with an approximate size of $10^{10}$ was used to pan against the soluble form of glycoprotein Hendra G (sG). The library was constructed by collecting peripheral blood B cells of 10 healthy donors. Seven unique Fabs isolated from the library demonstrated longer $V_H$ CDR3 among stronger binders compared to weakly bound Fabs. Isolated Fabs produced measurable inhibitory activity in reporter gene assay and inhibited syncytium formation. Later, conversion of a particular potent Fab to full antibody format exhibited 100% neutralizing potency with 12.5 μg/mL and 98% with 1.6 μg/mL [157].

*Candida albicans* is a very common pathogen to humans which usually attacks immunocompromised individuals with weak immune systems [158]. It has been reported that protein and carbohydrate moieties of *C. albicans* cell wall elicit strong immunological responses in humans [159]. Hence, isolation of antibodies which are able to bind to these moieties could lead to a better understanding of antibody reactivity towards these surface antigens for therapeutic purposes. A combinatorial phage display scFv library was used to pan against the surface antigen of *C. albicans*. The combinatorial library consists of two scFv libraries where one derived from lambda (λ) 2 family and another one derived from kappa (κ) 3 family. Panning of antigens used roughly $10^{12}$ from each phage library where panning with $V_L$ λ2 and $V_L$ κ3 library managed to obtain enrichments of 20-fold and 300-fold respectively. Three scFv clones were isolated and confirmed to bind with blastoconidia surface antigen via immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and western blot [160].

Malaria is a life-threatening disease caused by *Plasmodium* parasites that is transmitted by mosquitoes. Out of the five species, *Plasmodium falciparum* is the most common and lethal type which leads to morbidity and mortality [161]. *P. falciparum* histidine-rich protein 2 (PfHRP2) is a common candidate for the detection of *P. falciparum* infection which remains in the bloodstream up to 28 days upon infection. A naïve scFv library which was applied for isolation of antibodies against protective antigen PA83of *Bacillus anthracis* [140] mentioned earlier was also used to pan against PfHRP2. This highlights the flexibility afforded by naïve libraries to be used against various disease targets simultaneously.

## 3.4 Potential Applications of Antibodies Derived from Naïve Antibody Libraries

Monoclonal antibodies are recognised as an important class of drug especially against autoimmune diseases, cancers and infectious diseases [156, 166]. To accommodate the increased interest in biomedical applications of antibodies, antibody phage display technology has provided an alternative for rapid discovery and broad utilisation of novel, highly specific and fully human antibodies [167].

Since the introduction of monoclonal antibody technology by Kohler and Milstein in 1975, antibodies have become important biomolecules due to their capability to be applied in various fields, and most importantly in diagnostic, therapeutics, and vaccine development [168]. The potential of applications of naïve antibodies isolated from naïve libraries in diagnostics had been demonstrated in few publications. Isolated scFv and Fab antibody fragments against native spores of *Bacillus subtilis* IFO 3336 were labelled with two different fluorescent dyes (FITC or rhodamine-*N*-hydroxysuccinimide) for direct and indirect detection by fluorescence microscope. The detection signal was greatly amplified when fluorescent dye was labelled to the high-copy number of phage coat protein pVIII of the antibody phage particles. Direct detection of individual spores was made possible by fluorescent-labelled antibody-phage clones [139]. These spore binding antibodies could be further incorporated into other detection systems such as high-flow-rate fluidized bed as solid phase capture [169], electrochemiluminescent immunoassay (ECLIA) [170], and magnetic particle fluorogenic immunoassay (MPFIA) [170].

Another example was demonstrated by *L. acidophilus* species specific scFv isolated with phage display technology. The resulting scFvs isolated was applied in FACS for enrichment of *L. acidophilus* which resulted in more than 99.8% genome coverage. It was envisioned that this species specific antibodies could be applied to enrich low abundance organism in a community which could lead to better taxonomic identification and genome recovery prior to genome sequencing [143].

Conversion of antibody fragments into full IgG format or Fc format is always preferable for therapeutics [171]. Naïve Fab antibody fragments isolated against HA region of H5N1 virus were converted into full human IgG format. These antibodies showed binding to conserved region HA2 and exhibited promising neutralization efficacy [145]. Another publication reported potential therapeutic applications against Hendra virus and Nipah virus via conversion of the most potent naïve Fab into IgG1. Full-length IgG1 format has been shown to have much better cell fusion inhibitory activity with 50% lesser inhibitory concentration required [157]. The naïve scFv clones against 85B antigen of TB converted into scFv-Fc (yumab) format was able to serve as a potential biomarker for the diagnostic of TB. Validation of binding between this scFv-Fc antibody and 85B antigen was performed with titration ELISA and sandwich ELISA. The lowest detection limit achieved was 5 ng/mL and 10 ng/mL respectively. Later, these antibodies were put onto the platform of lateral flow immunoassay (LFIA) for detection of 85 B by conjugating the antibodies to 40 nm colloidal gold. The assay was able to detect the antigen at ≤ 5 ng/mL. Mtb culture filtrate was also successfully detected using immunoblot, analysed by reducing gel analysis via Tape station [141].

Despite the low instances of naïve antibody libraries being applied for infectious diseases, the antibodies derived from these libraries could be applied in a similar fashion for infectious disease therapy like those in oncology or autoimmunity. Antibodies can play a crucial role in neutralizing the infectivity of a virus by blocking binding sites or receptors on the virus or host cells. These class of antibodies are known as neutralizing antibodies (NAbs). Neutralization is defined as the reduction in viral infectivity via the binding of antibodies to viral surface particles which leads to the blocking of viral replication processes [172, 173]. Viral infection requires the attachment of the virus to the host cell membrane but different viruses may utilize different approaches for infection. Naked viruses (adenoviruses and papillomaviruses) are able to penetrate the host cell membrane to enter the cytoplasm or inject their genome through the membrane [174]. While enveloped viruses (dengue viruses, zika viruses, ebola viruses) have to fuse their envelopes with hosts' membranes for attachment [175–177]. Therefore, antibodies binding to influenza virus membrane protein is able to impede the fusion of influenza viruses with host's cell membrane [178].

Targeting viral envelope proteins of envelope viruses is becoming an attractive option for both diagnostic and therapy since the envelope proteins are expressed on viral surface and are therefore accessible for antibody attachment [179]. For example, dengue envelope (E) protein is a common target for neutralizing antibodies and also vaccine development since it contains few neutralising epitopes and binding motifs for virus entry into host cells [180]. A human Fab mAb 5J7 was shown to bind to all four dengue serotypes in enzyme-linked immunosorbent assay (ELISA) but could only neutralize dengue serotype 3. This mAb is able to neutralise 50% of dengue virus at nanogram level. Cryo-electron microscopy (cryo-EM) showed one Fab is able to bind across three E proteins and engage only domains that are crucial for infection. Moreover, the 5J7 antibody could prevent the virus from entering the host cells after attachment. The pre-attachment neutralization test showed 100% efficiency with 10 ng $\mu L^{-1}$ of antibodies [181].

In some cases, polyclonal antibodies work better in detection of viral proteins such as dengue NS2B protein. NS2B protein works as a cofactor for NS3 protease activity [182]. NS2B protein is small in size making it difficult to target when the whole virus particle is used. Polyclonal antibodies against NS2B proteins

were generated via gene or protein immunisation in mice. Western blotting and immunofluorescence assays confirmed the polyclonal antibodies generated are able to recognise both the native and denatured form of NS2B protein [183]. In certain cases such as ebola virus (EBOV) infections, an antibody cocktail is used as NAbs. Administration of antibody cocktails instead of single antibodies has shown promising results on non-human primates. Several antibody cocktails against EBOV were developed in recent years, which includes ZMAb [184], MB-003 [185], ZMapp [186] and the combination of mAB114 with mAb100 [187] that demonstrates successful therapeutic activity. ZMapp, the improved version of two antibody cocktails (MB-003 and ZMAb) reported 100% survival rate when administered to rhesus macaques despite the treatment being initiated only after 5 days of infection [186]. The antibodies in the cocktail are found to target several vulnerable sites on the glycoprotein (GP). Neutralizing antibodies target the base of GP while the non-neutralizing antibodies will bind to the glycan cap and mucin-like domains which functions as an external domain for the viral attachment and fusion [188–192]. This allows for a cumulative effect on the neutralization of virus replication and prevents virus survival. The latest antibody cocktail of mAb114 and mAb100 was successful in protecting nonhuman primates against the Ebola virus disease which includes viremia. The combination of both antibodies function to target different regions of the Ebola virus to improve its efficacy. The antibody mAb100 was found to recognize the base of the Ebola virus GP trimer which prevents access to the cathepsin-cleavage loop and prevents the proteolytic cleavage of the GP that is required for virus entry. However, mAb114 was able to interact with the glycan cap and inner chalice of the GP. The antibody remains associated to the glycan cap even after proteolytic removal. This aids to inhibit the binding of the cleaved GP to its receptor. The combination effect of the two antibodies provides the basis of virus neutralization and protection against the Ebola virus [187].

The cocktail approach by using several different antibodies with varying specificities and functionalities offer a broader coverage to prevent the escape of the virus [193]. Aside from EBOV infections, antibody cocktails are also being applied in other viral infectious diseases such as rabies virus [194], SARS coronavirus [195], HIV [196], hepatitis B virus [197] and other viruses [198]. The application of naïve antibody-cocktails is no longer something new, with the formulation and commercialization of different naïve antibody cocktails for separation of B cells and T cells from samples (Merck, BDbiosciences, Miltenyi Biotec). However, applications of naïve antibody cocktails in therapies especially infectious diseases has yet to be realised in clinical settings.

One of the major attractions in using antibodies for therapy is the ability of antibodies to induce cytotoxicity. Antibodies are able to lyse the target molecules by triggering the antibody dependent cell mediated cytotoxic (ADCC), or complement dependent cytotoxic (CDC) activity. ADCC uses immune-effector cells such as macrophages and natural killer cells while CDC function by activating a cascade of complement proteins [199]. As an example, Rituximab (Rituxan®) targeted against the pan-B-cell marker CD20 induces cell death [200]. In this regard, several other pathogens such as varicella, tetanus, Respiratory Syncytial Virus (RSV), rabies and Hepatitis B has been reported to show successful prophylactic use of antibodies in exposed individuals [43, 135]. In cases of immunodeficient or immunosuppressed individuals especially in HIV infected patients, antibody therapy can be a viable option for therapy because it can provide immunity to other infectious pathogens without giving rise to any further T-cell stimulation which promotes HIV growth. In addition, antibodies can be given to infections where no vaccines are available, for example, Ebola or Marburg viruses.

Antibody-drug conjugates (ADCs) are complexes of antibodies covalently joined with potent or cytotoxic drugs using various conjugation techniques. This conjugates are widely applied in cancer therapies due to their capability to differentiate healthy and disease state tissues [201]. In 2015, a novel antibody-antibiotic conjugate was developed to eliminate intracellular *S. aureus*,

one of the major causes of bacterial infection in the world. The anti-*S. aureus* antibodies were screened and carefully selected from peripheral blood of patients recovering from various *S. aureus* infections and linked to highly efficacious antibiotics via capthesin-cleavable linker. The conjugate eliminates *S. aureus* via two mechanisms: (1) engulf by host cells and let intracellular proteases cleave and release the antibiotic in active form; (2) binding of conjugate to bacterium and deliver antibiotic within phagosome to kill the bacterial. This conjugate was able to kill *S. aureus* inside human macrophages, endothelial and epithelial cell lines [202]. Naïve antibody-drug conjugate would be a new avenue for therapy, not only in infectious diseases but also other diseases like cancer and autoimmune diseases. Conjugating drug or other potent molecules with naïve antibodies might be a promising way to overcome the limitations possess by intact naïve antibodies and conventional antibiotic treatments. Naïve libraries will likely prove to be a promising diagnostics and therapeutics tool for infectious diseases in the coming future due to their great diversity and immune-system-independent nature which gives endless possibilities to isolate antibodies targeting a wide array of disease antigens [156].

## 3.5 Conclusion

Antibody based therapies have indeed been on a comeback trail after the disappointment of murine mAbs which provoke undesired immune response in humans upon administration. Despite a lot of mAbs have been approved for clinical usage, only one mAb (Palivizumab) has been approved for treatment of infectious disease. Antibody based therapies for infectious diseases are still underdeveloped and neglected due to the lack of research interest compared to cancer and other pandemic diseases. Immunised antibody libraries have always been preferred for the development of antibodies against infectious disease antigens. Skewed repertoires of immunised libraries allow isolation of high-affinity binding antibodies. However, with the rate of emerging and re-emerging infectious diseases, generation of disease specific immunised libraries is not a feasible option. Hence, a good quality naïve antibody library with a satisfactory size and diversity becomes an impeccable tool to generate antibodies against infectious diseases. Despite the antibodies isolated from naïve antibody libraries might not have satisfactory binding affinities, the advancement of molecular technology could be utilised to overcome these limitations. We believe, with the aid of newer technologies, better quality human naïve libraries could be constructed for the isolation of better quality antibodies to combat the infectious diseases. Naïve antibody phage libraries will have a foreseeable role to play in the journey to overcome the challenges posed by infectious agents.

**Acknowledgments** The authors would like to acknowledge the support from the Malaysian Ministry of Higher Education through the Higher Institution Centre of Excellence (HICoE) Grant (Grant No. 311/CIPPM/4401005). SKC would like to acknowledge support from USM Fellowship.

## References

1. Janeway CA, Travers P, Walport M, Shlomchik MJ (1997) Immunobiology: the immune system in health and disease, vol 1. Current Biology Singapore
2. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) The generation of antibody diversity
3. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001a) B-cell activation by armed helper T cells
4. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001b) The generation of diversity in immunoglobulins
5. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001c) Principles of innate and adaptive immunity
6. Medzhitov R (2007) Recognition of microorganisms and activation of the immune response. Nature 449(7164):819–826
7. Maddaly R, Pai G, Balaji S, Sivaramakrishnan P, Srinivasan L, Sunder SS, Paul SF (2010) Receptors and signaling mechanisms for B-lymphocyte activation, proliferation and differentiation--insights from both in vivo and in vitro approaches. FEBS Letters 584(24):4883–4894
8. Fulcher D, Basten A (1997) B cell life span: a review. Immunol Cell Biol 75(5):446–455

9. Rolink A, Melchers F (1993) Generation and regeneration of cells of the B-lymphocyte lineage. Curr Opin Immunol 5(2):207–217
10. Raff M, Alberts B, Lewis J, Johnson A, Roberts K (2002) Molecular biology of the cell, 4th edn. Garland Science, New York
11. DeFranco AL (2001) B lymphocytes: receptors. In: eLS. Wiley, Chichester. https://doi.org/10.1002/9780470015902.a0000914.pub3
12. Melchers F, Rolink A (1999) B-lymphocyte development and biology. Fundamental immunology 183
13. LeBien TW, Tedder TF (2008) B lymphocytes: how they develop and function. Blood 112(5):1570–1580. https://doi.org/10.1182/blood-2008-02-078071
14. Melchers F (2015) Checkpoints that control B cell development. J Clin Invest 125(6):2203–2210
15. Tonegawa S (1983) Somatic generation of antibody diversity. Nature 302(5909):575–581
16. Taussig M (1988) Molecular genetics of immunoglobulins. Immunology 64(Suppl 1):7
17. Mathonet P, Ullman CG (2013) The application of next generation sequencing to the understanding of antibody repertoires. Front Immunol 4(265.10):3389
18. Luning Prak ET, Monestier M, Eisenberg RA (2011) B cell receptor editing in tolerance and autoimmunity. Ann N Y Acad Sci 1217(1):96–121
19. Bassing CH, Swat W, Alt FW (2002) The mechanism and regulation of chromosomal V (D) J recombination. Cell 109(2):S45–S55
20. Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE (1992) RAG-1-deficient mice have no mature B and T lymphocytes. Cell 68(5):869–877
21. Shinkai Y, Lam K-P, Oltz EM, Stewart V, Mendelsohn M, Charron J, Datta M, Young F, Stall AM, Alt FW (1992) RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V (D) J rearrangement. Cell 68(5):855–867
22. Hwang JK, Alt FW, Yeap L-S (2015) Related mechanisms of antibody somatic hypermutation and class switch recombination. Microbiol Spectr 3(1):MDNA3-0037-2014
23. Di Noia JM, Neuberger MS (2007) Molecular mechanisms of antibody somatic hypermutation. Annu Rev Biochem 76:1–22
24. Li Z, Woo CJ, Iglesias-Ussel MD, Ronai D, Scharff MD (2004) The generation of antibody diversity through somatic hypermutation and class switch recombination. Genes Dev 18(1):1–11
25. Jacob J, Kelsoe G, Rajewsky K, Weiss U (1991) Intraclonal generation of antibody mutants in germinal centres. Nature 354(6352):389–392
26. Weigert MG, Cesari IM, Yonkovich SJ, Cohn M (1970) Variability in the lambda light chain sequences of mouse antibody. Nature 228:1045–1047
27. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 102(5):553–563
28. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, Catalan N, Forveille M, Dufourcq-Lagelouse R, Gennery A (2000) Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell 102(5):565–575
29. Chaudhuri J, Khuong C, Alt FW (2004) Replication protein A interacts with AID to promote deamination of somatic hypermutation targets. Nature 430(7003):992–998
30. Muramatsu M, Sankaranand V, Anant S, Sugai M, Kinoshita K, Davidson NO, Honjo T (1999) Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J Biol Chem 274(26):18470–18476
31. Fagarasan S, Kawamoto S, Kanagawa O, Suzuki K (2009) Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. Annu Rev Immunol 28:243–273
32. Stavnezer J, Guikema JE, Schrader CE (2008) Mechanism and regulation of class switch recombination. Annu Rev Immunol 26:261
33. Victora GD, Nussenzweig MC (2012) Germinal centers. Annu Rev Immunol 30:429–457
34. Harris RS, Kong Q, Maizels N (1999) Somatic hypermutation and the three R's: repair, replication and recombination. Mutat Res Rev Mutat Res 436(2):157–178
35. Rada C, Milstein C (2001) The intrinsic hypermutability of antibody heavy and light chain genes decays exponentially. EMBO J 20(16):4570–4576
36. Morgan HD, Dean W, Coker HA, Reik W, Petersen-Mahrt SK (2004) Activation-induced Cytidine Deaminase Deaminates 5-Methylcytosine in DNA and is expressed in pluripotent tissues IMPLICATIONS FOR EPIGENETIC REPROGRAMMING. J Biol Chem 279(50):52353–52360
37. Keim C, Kazadi D, Rothschild G, Basu U (2013) Regulation of AID, the B-cell genome mutator. Genes Dev 27(1):1–17
38. Rajewsky K, Forster I, Cumano A (1987) Evolutionary and somatic selection of the antibody repertoire in the mouse. Science 238(4830):1088–1094
39. Rogozin IB, Diaz M (2004) Cutting edge: DGYW/WRCH is a better predictor of mutability at G: C bases in Ig hypermutation than the widely accepted RGYW/WRCY motif and probably reflects a two-step activation-induced cytidine deaminase-triggered process. J Immunol 172(6):3382–3384
40. Pham P, Bransteitter R, Petruska J, Goodman MF (2003) Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation. Nature 424(6944):103–107
41. Yu K, Huang F-T, Lieber MR (2004) DNA substrate length and surrounding sequence affect the activation-induced deaminase activity at cytidine. J Biol Chem 279(8):6496–6500
42. Rada C, Williams GT, Nilsen H, Barnes DE, Lindahl T, Neuberger MS (2002) Immunoglobulin isotype

switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. Curr Biol 12(20):1748–1755
43. Casadevall A, Dadachova E, Pirofski L-a (2004) Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2(9):695–703
44. Plotkin SA (2008) Correlates of vaccine-induced immunity. Clin Infect Dis 47(3):401–409
45. Pulendran B, Ahmed R (2006) Translating innate immunity into immunological memory: implications for vaccine development. Cell 124(4):849–863
46. Chaudhuri J, Basu U, Zarrin A, Yan C, Franco S, Perlot T, Vuong B, Wang J, Phan RT, Datta A (2007) Evolution of the immunoglobulin heavy chain class switch recombination mechanism. Adv Immunol 94:157–214
47. Matthews AJ, Zheng S, DiMenna LJ, Chaudhuri J (2014) Regulation of immunoglobulin class-switch recombination: choreography of noncoding transcription, targeted DNA deamination, and long-range DNA repair. Adv Immunol 122:1
48. Xu Z, Zan H, Pone EJ, Mai T, Casali P (2012) Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol 12(7):517–531
49. Honjo T (2008) A memoir of AID, which engraves antibody memory on DNA. Nat Immunol 9(4):335–337
50. Wagner K, Stickings P, White J, Neal S, Crowcroft N, Sesardic D, Efstratiou A (2009) A review of the international issues surrounding the availability and demand for diphtheria antitoxin for therapeutic use. Vaccine 28(1):14–20
51. Köhler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497
52. Tjandra JL, Ramadi L, McKenzie IF (1990) Development of human anti-murine antibody (HAMA) response in patients. Immunol Cell Biol 68(6):367–376
53. Bradbury AR, Sidhu S, Dübel S, McCafferty J (2011) Beyond natural antibodies: the power of in vitro display technologies. Nat Biotechnol 29(3):245–254
54. Edwards BM, He M (2012) Evolution of antibodies in vitro by ribosome display. In: Antibody engineering: methods and protocols, 2nd edn. Humana Press, Totowa, pp 281–292
55. Benatuil L, Perez JM, Belk J, Hsieh C-M (2010) An improved yeast transformation method for the generation of very large human antibody libraries. Protein Eng Des Sel 23(4):155–159
56. Boder ET, Raeeszadeh-Sarmazdeh M, Price JV (2012) Engineering antibodies by yeast display. Arch Biochem Biophys 526(2):99–106
57. Rockberg J, Löfblom J, Hjelm B, Uhlén M, Ståhl S (2008) Epitope mapping of antibodies using bacterial surface display. Nat Methods 5(12):1039–1045
58. Spatola BN, Murray JA, Kagnoff M, Kaukinen K, Daugherty PS (2012) Antibody repertoire profiling using bacterial display identifies reactivity signatures of celiac disease. Anal Chem 85(2):1215–1222
59. Chin CF, Ler LW, Choong YS, Ong EBB, Ismail A, Tye GJ, Lim TS (2016) Application of streptavidin mass spectrometric immunoassay tips for immunoaffinity based antibody phage display panning. J Microbiol Methods 120:6–14
60. Frenzel A, Schirrmann T, Hust M (2016) Phage display-derived human antibodies in clinical development and therapy. In: MAbs, vol 7. Taylor & Francis, pp 1177–1194
61. Sidhu SS, Geyer CR (2015) Phage display in biotechnology and drug discovery. CRC Press, Boca Raton
62. Sumida T, Yanagawa H, Doi N (2012) In vitro selection of fab fragments by mRNA display and gene-linking emulsion PCR. J Nucleic Acids 2012:371379
63. Xiao L, Hung K-C, Takahashi TT, Joo K-I, Lim M, Roberts RW, Wang P (2013) Antibody-mimetic ligand selected by mRNA display targets DC-SIGN for dendritic cell-directed antigen delivery. ACS Chem Biol 8(5):967–977
64. Jakobovits A, Amado RG, Yang X, Roskos L, Schwab G (2007) From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. Nat Biotechnol 25(10):1134–1143
65. McCafferty J, Griffiths AD, Winter G, Chiswell DJ (1990) Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348(6301):552–554
66. Lim TS (2009) Parameters affecting phage display library design for improved generation of human antibodies. Freie Universität Berlin Berlin, Berlin
67. Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 228(4705):1315–1317
68. Breitling F, Dübel S, Seehaus T, Klewinghaus I, Little M (1991) A surface expression vector for antibody screening. Gene 104(2):147–153
69. Tikunova N, Morozova V (2009) Phage display on the base of filamentous bacteriophages: application for recombinant antibodies selection. Acta Nat 1(3):20
70. Bazan J, Całkosiński I, Gamian A (2012) Phage display—a powerful technique for immunotherapy: 1. Introduction and potential of therapeutic applications. Hum Vaccin Immunother 8(12):1817–1828
71. Hust M, Dübel S (2005) Phage display vectors for the in vitro generation of human antibody fragments. In: Immunochemical protocols. Springer, pp 71–96
72. Vodnik M, Zager U, Strukelj B, Lunder M (2011) Phage display: selecting straws instead of a needle from a haystack. Molecules 16(1):790–817
73. Kwaśnikowski P, Kristensen P, Markiewicz WT (2005) Multivalent display system on filamentous bacteriophage pVII minor coat protein. J Immunol Methods 307(1):135–143
74. Løset GÅ, Roos N, Bogen B, Sandlie I (2011b) Expanding the versatility of phage display II: improved affinity selection of folded domains on protein VII and IX of the filamentous phage. PLoS One 6(2):e17433

75. Løset GÅ, Bogen B, Sandlie I (2011a) Expanding the versatility of phage display I: efficient display of peptide-tags on protein VII of the filamentous phage. PLoS One 6(2):e14702
76. Kramer RA, Cox F, van der Horst M, van den Oudenrijn S, Bia J, Logtenberg T, de Kruif J (2003) A novel helper phage that improves phage display selection efficiency by preventing the amplification of phages without recombinant protein. Nucleic Acids Res 31(11):e59–e59
77. Shi B, Wang H, Guo S, Xu Y, Li Z, Gu J (2007) Protein III-based single-chain antibody phage display using bacterial cells bearing an additional genome of a gene-III-lacking helper phage. BioTechniques 42(6):760
78. Chasteen L, Ayriss J, Pavlik P, Bradbury A (2006) Eliminating helper phage from phage display. Nucleic Acids Res 34(21):e145–e145
79. Carmen S, Jermutus L (2002) Concepts in antibody phage display. Brief Funct Genomic Proteomic 1(2):189–203
80. Walter G, Konthur Z, Lehrach H (2001) High-throughput screening of surface displayed gene products. Comb Chem High Throughput Screen 4(2):193–205
81. Noppe W, Plieva F, Galaev IY, Pottel H, Deckmyn H, Mattiasson B (2009) Chromato-panning: an efficient new mode of identifying suitable ligands from phage display libraries. BMC Biotechnol 9(1):21
82. Skerra A, Pluckthun A (1988) Assembly of a functional immunoglobulin Fv fragment in Escherichia coli. Science 240(4855):1038
83. Mazor Y, Van Blarcom T, Iverson BL, Georgiou G (2008) E-clonal antibodies: selection of full-length IgG antibodies using bacterial periplasmic display. Nat Protoc 3(11):1766–1777
84. Dübel S, Reichert JM (2014) Handbook of therapeutic antibodies, vol 1. Wiley, Weinheim
85. Holt LJ, Herring C, Jespers LS, Woolven BP, Tomlinson IM (2003) Domain antibodies: proteins for therapy. Trends Biotechnol 21(11):484–490
86. Kügler J, Wilke S, Meier D, Tomszak F, Frenzel A, Schirrmann T, Dübel S, Garritsen H, Hock B, Toleikis L (2015) Generation and analysis of the improved human HAL9/10 antibody phage display libraries. BMC Biotechnol 15(1):10
87. Schofield DJ, Pope AR, Clementel V, Buckell J, Chapple SD, Clarke KF, Conquer JS, Crofts AM, Crowther SR, Dyson MR (2007) Application of phage display to high throughput antibody generation and characterization. Genome Biol 8(11):R254
88. Hust M, Frenzel A, Meyer T, Schirrmann T, Dübel S (2012) Construction of human naive antibody gene libraries. In: Antibody engineering: methods and protocols, 2nd edn. Humana Press, Totowa, pp 85–107
89. Tiller T, Schuster I, Deppe D, Siegers K, Strohner R, Herrmann T, Berenguer M, Poujol D, Stehle J, Stark Y (2013) A fully synthetic human Fab antibody library based on fixed VH/VL framework pairings with favorable biophysical properties. In: MAbs, vol 3. Taylor & Francis, pp 445–470
90. Bradbury AR, Marks JD (2004) Antibodies from phage antibody libraries. J Immunol Methods 290(1):29–49
91. Sotelo PH, Collazo N, Zuñiga R, Gutiérrez-González M, Catalán D, Ribeiro CH, Aguillón JC, Molina MC (2012) An efficient method for variable region assembly in the construction of scFv phage display libraries using independent strand amplification. In: MAbs, vol 4. Taylor & Francis, pp 542–550
92. Steinwand M, Droste P, Frenzel A, Hust M, Dübel S, Schirrmann T (2014) The influence of antibody fragment format on phage display based affinity maturation of IgG. In: MAbs, vol 1. Taylor & Francis, pp 204–218
93. Ponsel D, Neugebauer J, Ladetzki-Baehs K, Tissot K (2011) High affinity, developability and functional size: the holy grail of combinatorial antibody library generation. Molecules 16(5):3675–3700
94. Lim BN, Chin CF, Choong YS, Ismail A, Lim TS (2016) Generation of a naïve human single chain variable fragment (scFv) library for the identification of monoclonal scFv against Salmonella Typhi Hemolysin E antigen. Toxicon 117:94–101
95. Miersch S, Sidhu S (2012) Synthetic antibodies: concepts, potential and practical considerations. Methods 57(4):486–498
96. Moon SA, Ki MK, Lee S, Hong M-L, Kim M, Kim S, Chung J, Rhee SG, Shim H (2011) Antibodies against non-immunizing antigens derived from a large immune scFv library. Mol Cells 31(6):509–513
97. Frenzel A, Kügler J, Wilke S, Schirrmann T, Hust M (2014) Construction of human antibody gene libraries and selection of antibodies by phage display. Human monoclonal antibodies: methods and protocols:215–243
98. Hutchings C, Carmen S, Lennard S (2001) Generation of naive human antibody libraries. In: Antibody engineering. Springer, pp 93–108
99. Sommavilla R, Lovato V, Villa A, Sgier D, Neri D (2010) Design and construction of a naive mouse antibody phage display library. J Immunol Methods 353(1):31–43
100. Zhu Z, Dimitrov DS (2009) Construction of a large naive human phage-displayed fab library through one-step cloning, Therapeutic Antibodies: Methods and Protocols, pp 129–142
101. Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR (1994) Making antibodies by phage display technology. Annu Rev Immunol 12(1):433–455
102. de Haard HJ, van Neer N, Reurs A, Hufton SE, Roovers RC, Henderikx P, de Brüine AP, Arends J-W, Hoogenboom HR (1999) A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. J Biol Chem 274(26):18218–18230

103. Aughan T, Willams A, Prichard K (2000) Human antibodies with sub2 nanomolar affinities isolated from a large non2immunized phage display. Nat Biotechnol 14:1149
104. Ling MM (2003) Large antibody display libraries for isolation of high-affinity antibodies. Comb Chem High Throughput Screen 6(5):421–432
105. Little M (2009) Recombinant antibodies for immunotherapy. Cambridge University Press, New York
106. Miertus S, Fassina G (2005) Combinatorial chemistry and technologies: methods and applications. CRC Press, Boca Raton
107. Beerli RR, Rader C (2010) Mining human antibody repertoires. MAbs 2(4):365–378
108. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G (1991) By-passing immunization: human antibodies from V-gene libraries displayed on phage. J Mol Biol 222(3):581–597
109. Ademokun A, YC W, Martin V, Mitra R, Sack U, Baxendale H, Kipling D, Dunn-Walters DK (2011) Vaccination-induced changes in human B-cell repertoire and pneumococcal IgM and IgA antibody at different ages. Aging Cell 10(6):922–930
110. Galson JD, Pollard AJ, Trück J, Kelly DF (2014) Studying the antibody repertoire after vaccination: practical applications. Trends Immunol 35(7):319–331
111. Brockmann E-C (2010) Evolution of bioaffinity reagents by phage display
112. Galson JD, Trück J, Fowler A, Clutterbuck EA, Münz M, Cerundolo V, Reinhard C, van der Most R, Pollard AJ, Lunter G (2015) Analysis of B cell repertoire dynamics following hepatitis B vaccination in humans, and enrichment of vaccine-specific antibody sequences. EBioMedicine 2(12):2070–2079
113. Dunn-Walters DK, Ademokun AA (2010) B cell repertoire and ageing. Curr Opin Immunol 22(4):514–520
114. Weksler ME (2000) Changes in the B-cell repertoire with age. Vaccine 18(16):1624–1628
115. Glanville J, Kuo TC, von Büdingen H-C, Guey L, Berka J, Sundar PD, Huerta G, Mehta GR, Oksenberg JR, Hauser SL (2011) Naive antibody gene-segment frequencies are heritable and unaltered by chronic lymphocyte ablation. Proc Natl Acad Sci 108(50):20066–20071
116. Shih H-Y, Krangel MS (2013) Chromatin architecture, CCCTC-binding factor, and V (D) J recombination: managing long-distance relationships at antigen receptor loci. J Immunol 190(10):4915–4921
117. Fuss IJ, Kanof ME, Smith PD, Zola H (2009) Isolation of whole mononuclear cells from peripheral blood and cord blood. Wiley Online Library
118. Sblattero D, Bradbury A (1998) A definitive set of oligonucleotide primers for amplifying human V regions. Immunotechnology 3(4):271–278
119. Tomlinson IM, Walter G, Marks JD, Llewelyn MB, Winter G (1992) The repertoire of human germline VH sequences reveals about fifty groups of VH segments with different hypervariable loops. J Mol Biol 227(3):776–798
120. Schwimmer LJ, Huang B, Giang H, Cotter RL, Chemla-Vogel DS, Dy FV, Tam EM, Zhang F, Toy P, Bohmann DJ (2013) Discovery of diverse and functional antibodies from large human repertoire antibody libraries. J Immunol Methods 391(1):60–71
121. Hust M, Meyer T, Voedisch B, Rülker T, Thie H, El-Ghezal A, Kirsch MI, Schütte M, Helmsing S, Meier D, Schirrmann T, Dubel S (2011) A human scFv antibody generation pipeline for proteome research. J Biotechnol 152(4):159–170
122. Lee C-H, Lee Y-C, Liang M-H, Leu S-J, Lin L-T, Chiang J-R, Yang Y-Y (2016) Antibodies against venom of the snake Deinagkistrodon acutus. Appl Environ Microbiol 82(1):71–80
123. Lin A, Jimenez J, Derr J, Vera P, Manapat ML, Esvelt KM, Villanueva L, Liu DR, Chen IA (2011) Inhibition of bacterial conjugation by phage M13 and its protein g3p: quantitative analysis and model. PLoS One 6(5):e19991
124. Høydahl LS, Nilssen NR, Gunnarsen KS, du Pré MF, Iversen R, Roos N, Chen X, Michaelsen TE, Sollid LM, Sandlie I (2016) Multivalent pIX phage display selects for distinct and improved antibody properties. Sci Rep 6:39066
125. Phipps ML, Lillo AM, Shou Y, Schmidt EN, Paavola CD, Naranjo L, Bemdich S, Swanson BI, Bradbury AR, Martinez JS (2016) Beyond helper phage: using "helper cells" to select peptide affinity ligands. PLoS One 11(9):e0160940
126. Nian S, Wu T, Ye Y, Wang X, Xu W, Yuan Q (2016) Development and identification of fully human scFv-Fcs against Staphylococcus aureus. BMC Immunol 17(1):1
127. Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, McCafferty J, Hodits RA, Wilton J, Johnson KS (1996) Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. Nat Biotechnol 14(3):309–314
128. Arbabi-Ghahroudi M, Tanha J, MacKenzie R (2009) Isolation of monoclonal antibody fragments from phage display libraries. In: Bacteriophages: methods and protocols, Volume 2 Molecular and Applied Aspects: 341–364. Humana Press, New York
129. Butler CD (2012) Infectious disease emergence and global change: thinking systemically in a shrinking world. Infect Dis Poverty 1(1):1
130. Morse SS (2001) Factors in the emergence of infectious diseases. In: Plagues and politics. Springer, pp 8–26
131. Hey A (2015) History and practice: antibodies in infectious diseases. Microbiol Spectr 3(2)
132. Casadevall A, Pirofski L-A (2015) The Ebola epidemic crystallizes the potential of passive antibody therapy for infectious diseases. PLoS Pathog 11(4):e1004717–e1004717

133. Lim BN, Tye GJ, Choong YS, Ong EBB, Ismail A, Lim TS (2014) Principles and application of antibody libraries for infectious diseases. Biotechnol Lett 36(12):2381–2392
134. Casadevall A (1996) Antibody-based therapies for emerging infectious diseases. Emerg Infect Dis 2(3):200
135. Keller MA, Stiehm ER (2000) Passive immunity in prevention and treatment of infectious diseases. Clin Microbiol Rev 13(4):602–614
136. Ong EBB, Anthony AA, Ismail A, Ismail A, Lim TS (2013) Cloning, expression, and purification of the hemolysin/cytolysin (HlyE antigen) from Salmonella enterica serovar Typhi: potential application for immunoassay development. Diagn Microbiol Infect Dis 77(1):87–89
137. Borio L, Frank D, Mani V, Chiriboga C, Pollanen M, Ripple M, Ali S, DiAngelo C, Lee J, Arden J (2001) Death due to bioterrorism-related inhalational anthrax: report of 2 patients. JAMA 286(20):2554–2559
138. Bush LM, Abrams BH, Beall A, Johnson CC (2001) Index case of fatal inhalational anthrax due to bioterrorism in the United States. N Engl J Med 345(22):1607–1610
139. Zhou B, Wirsching P, Janda KD (2002) Human antibodies against spores of the genus Bacillus: a model study for detection of and protection against anthrax and the bioterrorist threat. Proc Natl Acad Sci 99(8):5241–5246
140. Cirino NM, Sblattero D, Allen D, Peterson SR, Marks JD, Jackson PJ, Bradbury A, Lehnert BE (1999) Disruption of anthrax toxin binding with the use of human antibodies and competitive inhibitors. Infect Immun 67(6):2957–2963
141. Fuchs M, Kämpfer S, Helmsing S, Spallek R, Oehlmann W, Prilop W, Frank R, Dübel S, Singh M, Hust M (2014) Novel human recombinant antibodies against Mycobacterium tuberculosis antigen 85B. BMC Biotechnol 14(1):1
142. Zaman K (2010) Tuberculosis: a global health problem. J Health Popul Nutr 28:111–113
143. Close DW, Ferrara F, Dichosa AE, Kumar S, Daughton AR, Daligault HE, Reitenga KG, Velappan N, Sanchez TC, Iyer S (2013) Using phage display selected antibodies to dissect microbiomes for complete de novo genome sequencing of low abundance microbes. BMC Microbiol 13(1):1
144. Chen H, Smith G, Li K, Wang J, Fan X, Rayner J, Vijaykrishna D, Zhang J, Zhang L, Guo C (2006) Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc Natl Acad Sci U S A 103(8):2845–2850
145. Lim AP, Chan CE, Wong SK, Chan AH, Ooi EE, Hanson BJ (2008) Neutralizing human monoclonal antibody against H5N1 influenza HA selected from a Fab-phage display library. Virol J 5(1):1
146. Donà MG, Giorgi C, Accardi L (2007) Characterization of antibodies in single-chain format against the E7 oncoprotein of the human papillomavirus type 16 and their improvement by mutagenesis. BMC Cancer 7(1):1
147. Rodríguez-Díaz J, Monedero V, Pérez-Martínez G, Buesa J (2004) Single-chain variable fragment (scFv) antibodies against rotavirus NSP4 enterotoxin generated by phage display. J Virol Methods 121(2):231–238
148. van den Brink EN, ter Meulen J, Cox F, Jongeneelen MA, Thijsse A, Throsby M, Marissen WE, Rood PM, Bakker AB, Gelderblom HR (2005) Molecular and biological characterization of human monoclonal antibodies binding to the spike and nucleocapsid proteins of severe acute respiratory syndrome coronavirus. J Virol 79(3):1635–1644
149. Bollati M, Alvarez K, Assenberg R, Baronti C, Canard B, Cook S, Coutard B, Decroly E, de Lamballerie X, Gould EA (2010) Structure and functionality in flavivirus NS-proteins: perspectives for drug design. Antivir Res 87(2):125–148
150. Lescar J, Luo D, Xu T, Sampath A, Lim SP, Canard B, Vasudevan SG (2008) Towards the design of antiviral inhibitors against flaviviruses: the case for the multifunctional NS3 protein from Dengue virus as a target. Antivir Res 80(2):94–101
151. Moreland NJ, Tay MY, Lim E, Paradkar PN, Doan DN, Yau YH, Shochat SG, Vasudevan SG (2010) High affinity human antibody fragments to dengue virus non-structural protein 3. PLoS Negl Trop Dis 4(11):e881
152. Zhao Y, Moreland NJ, Tay MY, Lee CC, Swaminathan K, Vasudevan SG (2014) Identification and molecular characterization of human antibody fragments specific for dengue NS5 protein. Virus Res 179:225–230
153. Hu D, Hu S, Wan W, Xu M, Du R, Zhao W, Gao X, Liu J, Liu H, Hong J (2015) Effective optimization of antibody affinity by phage display integrated with high-throughput DNA synthesis and sequencing technologies. PLoS One 10(6):e0129125
154. Kabir ME, Krishnaswamy S, Miyamoto M, Furuichi Y, Komiyama T (2009) An improved phage-display panning method to produce an HM-1 killer toxin anti-idiotypic antibody. BMC Biotechnol 9(1):1
155. Kristensen P, Winter G (1998) Proteolytic selection for protein folding using filamentous bacteriophages. Fold Des 3(5):321–328
156. Pansri P, Jaruseranee N, Rangnoi K, Kristensen P, Yamabhai M (2009) A compact phage display human scFv library for selection of antibodies to a wide variety of antigens. BMC Biotechnol 9(1):6. https://doi.org/10.1186/1472-6750-9-6
157. Zhu Z, Dimitrov AS, Bossart KN, Crameri G, Bishop KA, Choudhry V, Mungall BA, Feng Y-R, Choudhary A, Zhang M-Y (2006) Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. J Virol 80(2):891–899
158. Kim J, Sudbery P (2011) Candida albicans, a major human fungal pathogen. J Microbiol 49(2):171–177

159. Martínez JP, Gil ML, López-Ribot JL, Chaffin WL (1998) Serologic response to cell wall mannoproteins and proteins of Candida albicans. Clin Microbiol Rev 11(1):121–141
160. Haidaris CG, Malone J, Sherrill LA, Bliss JM, Gaspari AA, Insel RA, Sullivan MA (2001) Recombinant human antibody single chain variable fragments reactive with Candida albicans surface antigens. J Immunol Methods 257(1):185–202
161. Ogunseitan O (2011) Green health: an A-to-Z guide, vol 9. Sage, Thousand Oaks
162. Kirsch MI, Hülseweh B, Nacke C, Rülker T, Schirrmann T, Marschall H-J, Hust M, Dübel S (2008) Development of human antibody fragments using antibody phage display for the detection and diagnosis of Venezuelan equine encephalitis virus (VEEV). BMC Biotechnol 8(1):66
163. Moghaddam A, Løbersli I, Gebhardt K, Braunagel M, Marvik OJ (2001) Selection and characterisation of recombinant single-chain antibodies to the hapten Aflatoxin-B1 from naive recombinant antibody libraries. J Immunol Methods 254(1):169–181
164. Schütte M, Thullier P, Pelat T, Wezler X, Rosenstock P, Hinz D, Kirsch MI, Hasenberg M, Frank R, Schirrmann T (2009) Identification of a putative Crf splice variant and generation of recombinant antibodies for the specific detection of Aspergillus fumigatus. PLoS One 4(8):e6625
165. Leow CH, Jones M, Cheng Q, Mahler S, McCarthy J (2014) Production and characterization of specific monoclonal antibodies binding the Plasmodium falciparum diagnostic biomarker, histidine-rich protein 2. Malar J 13(1):1
166. Breedveld F (2000) Therapeutic monoclonal antibodies. Lancet 355(9205):735–740
167. Schirrmann T, Meyer T, Schütte M, Frenzel A, Hust M (2011) Phage display for the generation of antibodies for proteome research, diagnostics and therapy. Molecules 16(1):412–426
168. Nissim A, Chernajovsky Y (2008) Historical development of monoclonal antibody therapeutics. In: Therapeutic antibodies. Springer, Berlin, pp 3–18
169. Weimer BC, Walsh M, Beer C, Koka R, Wang X (2001) Solid-phase capture of proteins, spores, and bacteria. Appl Environ Microbiol 67(3):1300–1307
170. Yu H (1998) Comparative studies of magnetic particle-based solid phase fluorogenic and electrochemiluminescent immunoassay. J Immunol Methods 218(1):1–8
171. Chames P, Van Regenmortel M, Weiss E, Baty D (2009) Therapeutic antibodies: successes, limitations and hopes for the future. Br J Pharmacol 157(2):220–233
172. Dimmock NJ (2012) Neutralization of animal viruses, vol 183. Springer, Berlin
173. Klasse P, Sattentau Q (2002) Occupancy and mechanism in antibody-mediated neutralization of animal viruses. J Gen Virol 83(9):2091–2108
174. Suomalainen M, Greber UF (2013) Uncoating of non-enveloped viruses. Curr Opin Virol 3(1):27–33
175. Pierson TC, Kielian M (2013) Flaviviruses: braking the entering. Curr Opin Virol 3(1):3–12
176. Plattet P, Plemper RK (2013) Envelope protein dynamics in paramyxovirus entry. MBio 4(4):e00413–e00413
177. Sun X, Whittaker GR (2006) Entry of influenza virus. In: Viral entry into host cells. Springer, New York, pp 72–82
178. Friesen RH, Koudstaal W, Koldijk MH, Weverling GJ, Brakenhoff JP, Lenting PJ, Stittelaar KJ, Osterhaus AD, Kompier R, Goudsmit J (2010) New class of monoclonal antibodies against severe influenza: prophylactic and therapeutic efficacy in ferrets. PLoS One 5(2):e9106
179. Teissier E, Penin F, Pécheur E-I (2010) Targeting cell entry of enveloped viruses as an antiviral strategy. Molecules 16(1):221–250
180. Roehrig JT, Bolin RA, Kelly RG (1998) Monoclonal antibody mapping of the envelope glycoprotein of the dengue 2 virus, Jamaica. Virology 246(2):317–328
181. Fibriansah G, Tan JL, Smith SA, De Alwis R, Ng T-S, Kostyuchenko VA, Jadi RS, Kukkaro P, de Silva AM, Crowe JE (2015) A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins. Nat Commun 6:6341
182. Zuo Z, Liew OW, Chen G, Chong PCJ, Lee SH, Chen K, Jiang H, Puah CM, Zhu W (2009) Mechanism of NS2B-mediated activation of NS3pro in dengue virus: molecular dynamics simulations and bioassays. J Virol 83(2):1060–1070
183. Liu L, Tian Y, Gao N, Chen Z, Zhang H, An J (2010) Application of antibodies against nonstructural protein 2B of dengue serotype 2 virus induced by DNA immunisation or recombinant protein NS 2B immunisation in BALB/c mice. J Virol Methods 163(1):10–16
184. Qiu X, Audet J, Wong G, Pillet S, Bello A, Cabral T, Strong JE, Plummer F, Corbett CR, Alimonti JB (2012) Successful treatment of Ebola virus–infected cynomolgus macaques with monoclonal antibodies. Sci Transl Med 4(138):138ra181–138ra181
185. Pettitt J, Zeitlin L, Kim DH, Working C, Johnson JC, Bohorov O, Bratcher B, Hiatt E, Hume SD, Johnson AK (2013) Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. Sci Transl Med 5(199):199ra113–199ra113
186. Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, Fausther-Bovendo H, Wei H, Aviles J, Hiatt E (2014) Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature 514:47–53
187. Corti D, Misasi J, Mulangu S, Stanley DA, Kanekiyo M, Wollen S, Ploquin A, Doria-Rose NA, Staupe RP, Bailey M (2016) Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. Science 351(6279):1339–1342
188. Davidson E, Bryan C, Fong RH, Barnes T, Pfaff JM, Mabila M, Rucker JB, Doranz BJ (2015) Mechanism

of binding to Ebola virus glycoprotein by the ZMapp, ZMAb, and MB-003 cocktail antibodies. J Virol 89(21):10982–10992
189. Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, Saphire EO (2008) Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature 454(7201):177–182
190. Lee JE, Saphire EO (2009) Neutralizing ebolavirus: structural insights into the envelope glycoprotein and antibodies targeted against it. Curr Opin Struct Biol 19(4):408–417
191. Murin CD, Fusco ML, Bornholdt ZA, Qiu X, Olinger GG, Zeitlin L, Kobinger GP, Ward AB, Saphire EO (2014) Structures of protective antibodies reveal sites of vulnerability on Ebola virus. Proc Natl Acad Sci 111(48):17182–17187
192. Wilson JA, Hevey M, Bakken R, Guest S, Bray M, Schmaljohn AL, Hart MK (2000) Epitopes involved in antibody-mediated protection from Ebola virus. Science 287(5458):1664–1666
193. Both L, Banyard AC, van Dolleweerd C, Wright E, Ma JK-C, Fooks AR (2013) Monoclonal antibodies for prophylactic and therapeutic use against viral infections. Vaccine 31(12):1553–1559
194. Goudsmit J, Marissen WE, Weldon WC, Niezgoda M, Hanlon CA, Rice AB, de Kruif J, Dietzschold B, Bakker AB, Rupprecht CE (2006) Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin. J Infect Dis 193(6):796–801
195. Ter Meulen J, Van Den Brink EN, Poon LL, Marissen WE, Leung CS, Cox F, Cheung CY, Bakker AQ, Bogaards JA, Van Deventer E (2006) Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. PLoS Med 3(7):e237
196. Armbruster C, Stiegler GM, Vcelar BA, Jäger W, Köller U, Jilch R, Ammann CG, Pruenster M, Stoiber H, Katinger HW (2004) Passive immunization with the anti-HIV-1 human monoclonal antibody (hMAb) 4E10 and the hMAb combination 4E10/2F5/2G12. J Antimicrob Chemother 54(5):915–920
197. Sawada H, Iwasa S, Nishimura O, Kitano K (1995) Efficient production of anti-(hepatitis B virus) antibodies and their neutralizing activity in chimpanzees. Appl Microbiol Biotechnol 43(3):445–451
198. Prabakaran M, Prabhu N, He F, Hongliang Q, Ho H-T, Qiang J, Goutama M, Kwang J (2009) Combination therapy using chimeric monoclonal antibodies protects mice from lethal H5N1 infection and prevents formation of escape mutants. PLoS One 4(5):e5672
199. Zafir-Lavie I, Michaeli Y, Reiter Y (2007) Novel antibodies as anticancer agents. Oncogene 26(25):3714–3733
200. Cerny T, Borisch B, Introna M, Johnson P, Rose AL (2002) Mechanism of action of rituximab. Anti-Cancer Drugs 13:S3–S10
201. Chudasama V, Maruani A, Caddick S (2016) Recent advances in the construction of antibody-drug conjugates. Nat Chem 8(2):114–119
202. Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, DePalatis L, Raab H, Hazenbos WL, Morisaki JH (2015) Novel antibody–antibiotic conjugate eliminates intracellular S. aureus. Nature 527(7578):323–328