Review Article

Therapeutic Proteins through Phage Display- A Brilliant Technique for its Simplicity

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ABSTRACT

Background: Phage Display Technology is an advanced version of rDNA and hybridoma technologies with simplicity in its procedure and production of desired therapeutics and biocontrol products. In this context, the filamentous bacteriophage namely M13 phage was explored by the Nobel Laureates, George P Smith and Gregory P Winter. Due to the unique tolerance of M13 phage to experimental conditions compared to the tailored B-cells, the global bacteriophage mediated pharma market is at its up-surge. In the present mini-review the sequential breakthroughs in the production of therapeutic antibodies and their market potential are explicitly shown. The use of phage for domestic and therapeutic applications needs to be popularized. **Materials and Methods:** In the present mini review, it is aimed to project the importance of phage display technology over the conventional hybridoma technology. The pertinent literature pertaining to the products of phage display and the scope to enhance the same are considered. **Results and Conclusion:** Both rDNA technology and hybridoma technology introduced for the first time to the scientific community a tool to design customized monoclonal antibodies using tailored hybrid B-cells and revolutionized medical diagnosis and treatment. They constituted 53% of approved biopharmaceuticals worth billions of US$. They qualify 8th position out of top 10 bestselling pharma products. Alternatively, the emerging technology in pharma industry namely Phage Display for the production of peptides and antibody formats compatible to the host systems came into vogue and revolutionized pharma market and yet to penetrate in Indian pharma industry.

Key words: Phage Display, M13, George P Smith, Sir Gregory P Winter, Pharma Market.

INTRODUCTION

A number of proteins namely monoclonal antibodies, globulins, albumins, enzymes, hormones, cytokines etc are being used to cure various illnesses. These biological macromolecules are normally not manufactured in pharma-based chemical industries, as they can make only small ligand molecules as leads or drugs. Whereas, a large many bio-based pharma industries through bioreactors and fermenters, adopting recombinant immortal cell lines, hybrid cells, yeast cells, bacteria and bacteriophages are manufacturing biologicals of therapeutic importance. Since the time recombinant DNA technology came into being, therapeutic proteins are gradually piling up in the health market.[1,2] rDNA technology invented by Paul Berg in 1959 involves a few molecular ingredients such as restriction enzymes, multiple cloning sites, ligases, plasmid vectors, screening and gene of interest. Paul Berg was the recipient of the Nobel Prize in the year 1980 in Chemistry. Further, the advent of hybridoma technology was in the year 1975 by George Kohler and Cesar Milstein. They were rewarded Nobel Prize in the year 1984. Hybridoma technology gave for the first time to the scientific community a tool to design customized monoclonal antibodies using tailored hybrid B-cells primarily for the purpose of diagnosis and treatment.[3] In consequence, a variety of

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developments led in reshaping monoclonal antibodies as chimeric antibodies, abzymes, humanized antibodies, diagnostic antibodies coupled with dyes, therapeutic antibodies coupled with drugs etc., had come into vogue.[4-7] The aforementioned two techniques dictated the production of a variety of therapeutic proteins in bio-based pharma industries since the year 1980 and served the humanity to resolve several health issues. However, of late, the yield of therapeutic macromolecules, down-stream processing and precise sterile protocols are all becoming economically unviable and unreachable to the rural mass. Further, the hybridoma technology is murine based. The first therapeutic product developed using this technique namely muromonab (OKT3) was designed to block CD3 of activated Tc cells so as to prevent graft rejection.[8] However, the same elicited antibody response in humans. To nullify the same, the chimeric antibodies were designed namely rituximab wherein the CH3 domain of mouse IgG was replaced with CH3 domain of human IgG.[9] Thus, humanized antibodies came into vogue. A few to mention here are declizumab[10] and bevacizumab.[11] The $F(ab)_2$ domain of humanized antibodies are still not found safe as its CDRs act as antigens due to its non-human origin. There is a good market potential in both diagnostics and therapeutics for these monoclonal antibodies and their sales rose up to 75 million US$ in the year 2013,[12] hence the need is felt to engineer exclusively human antibodies which elicit least immunogenicity.

In this context, a new technique that has been under trials since the year 1985, namely phage display, has now revolutionized the massive production of biological macromolecules within the affordable cost. George P Smith, University of Missouri, USA and Sir Gregory P Winter, University of Cambridge, UK were the recipients of the Nobel Prize in Chemistry in the year 2018 for their contribution in the field of "Phage Display of Peptides and Antibodies" along with Frances H Arnold, University of California, USA for her study on "Directed Evolution of Enzymes". Phage display has turned out to be a major technology breakthrough wherein the desired packaged rDNA is allowed to transcribe a specific macromolecular protein which will be displayed as a fusion coat protein on the surface of a bacteriophage (Figure 1). In consequence, the phage display technique is explored to serve as a platform in allowing and screening a number of target binding proteins having highest affinity for specific targets. Therefore, in the present article, the author is attempting to elucidate in nutshell the sequential scientific breakthroughs; each is an advanced version of the previous technology that eventually helped humanity by providing affordable therapeutic biological macromolecules.

Immune checkpoint therapy

James P Allison, popularly known in the scientific community as Jim Allison focussed on basic biological inventions of the master immune T cells and blockade of its immune checkpoint to accentuate treatment for cancer. In his exploration, he has chosen one of the T-cell transmembrane proteins CTLA-4, which inherently negatively regulates Tc cell function upon binding to B7 and hence acts as a break to restrain from the full and everlasting potential of activation. His aim is to prevent negative regulation of Tc by blocking CTLA-4 so as to make Tc free to attack cancer cell.[13] He is greatly succeeded and rewarded. A monoclonal antibody (Ipilimumab) against CTLA-4 is developed by Jim to block CTLA-4 from binding to B7 receptor of APC and free from negative regulation. This experimented ipilimumab is approved in a fast-track procedure by U.S. Food and Drug Administration in 2011. Clinical trials using this immune therapy technique are in progress in several other cancer types. Ultimately, Jim's research has led to "life-saving treatment" for ill people who otherwise have had no hope. Simultaneously yet another Nobel Laureate Tasuku Honjo, a Japanese immunologist, discovered the mechanism and proteins related to the regulation of Tc cell immune responses and the same led to the development of novel immunotherapies against cancers such as melanoma. Honjo was recognized by the Assembly of Karolinska Institute in Stockholm for his investigations in immunotherapies against human patients suffering from melanoma in the 2018 Nobel Prize for Physiology or Medicine. Professor Tasuku Honjo shared this award with James P. Allison, who deduced yet another different pathway to kill even advanced cancer cells.

In the early 2000s, Honjo hypothesized that inhibition of PD-1 in laboratory animal models of cancer uniquely restores the immune potential of Tc cells to target and kill cancer cells. In this journey, Tasuku Honjo and his colleagues, from the Department of Medical Chemistry at Kyoto University, Japan, discovered a PD-1 on the surface of Tc cell. In later experiments, he deduced its function as a negative regulator of immune response similar to CTLA-4. Honjo showed that PD-1 of Tc cell binds to its corresponding ligand molecules PD-L1 and PD-L2 produced by cancer cells.[14] This binding literally stops Tc-cell function and causes them to self-destruct. Thus, through this strategy, cancer cells cleverly evade the immune surveillance. Honjo focused on blocking PD-1 on Tc cells with an antibody in animal models of

cancer and thus disallowed molecules (PD-L-1) from cancer cells to bind. This attempt has shown interestingly that Tc-cells retained their potential to target and kill cancer cells. Honjo's discoveries led to the development of novel anti-PD-1 cancer immunotherapies namely nivolumab and pembrolizumab. Nivolumab, marketed as Opdivo, is a humanized IgG4 anti-PD-1 monoclonal antibody. It is approved for the treatment of melanoma, a skin cancer.

The 2018 Nobel Prize in Physiology or Medicine was bestowed on Allison and Honjo, who independently established and unravelled how the two different strategies for blocking the checkpoints on the immune system particularly Tc cells are used in the treatment of patients suffering from cancer illness. Their seminal discoveries showcased a new principle and procedure for cancer therapy and a landmark in the fight against cancer. This episode paves the way for the enhanced requirement of phage-derived antibody formats.

Phage Display

Phage display is a technique created and propagated by George Smith.[15] This technique is now extensively used for the production of biopharmaceuticals to neutralize toxins and antigens. George Smith started, in the first half of 1980s, to use viruses that infect bacteria with the hope that these bacteriophages, being simple in their genetic construction, are employed to clone genes. His incipient idea is to clone unknown genes to express known proteins. Later, Smith extended his study so as to make the phage to carry genes of known therapeutic proteins namely antibodies. In compliance with his expectation, antibodies produced by a phage could precisely bind to their corresponding epitopes of antigens. In this elegant attempt, he could track the phage that he constructed out of a mixture of innumerable phage particles.[16,17] Through this experiment, George Smith laid a foundation of what it has come to be known as "Phage Display" and this elegant protocol was rewarded by the Royal Swedish Academy of Sciences, Stockholm in the year 2018 along with Greg Winter, whose contributions were on phage antibody pharmaceuticals. This technique is brilliant in its simplicity and hence became popular in a short time.

Phage as a tool

A few bacteriophages *viz.*, Lambda, T4, T7 and Ff filamentous phages (M13, fl and fd) are being used as tools for phage display as they are reliable cloning vectors. The best characterized among these cloning vehicles are M13, fl and fd as they infect *Escherichia coli* containing F-conjugate plasmid. M13 filamentous phage libraries are available from New England Biolabs. In addition, hybrid phage, helper phage and hyper phage are employed to enhance the display of fusion folded globular proteins/peptides.[18] The hybrid phage contains wild type genome and a copy of fusion gene. The helper phage containing defective origin of replication (M13VCS or M13K07), is yet another engineered bacteriophage required for phage assembly/packaging into M13 particle.[19] A modified helper phage known as hyper phage has wild type pIII phenotype which enhances the yield of recombinant fusion proteins and also used for integration into phage particles. The hyperphage carries functional pIII on their surface. The filamentous phage infects gram-negative bacteria possessing bacterial pili that serve as corresponding receptors and yields titres up to 10^{13} /ml bacterial culture.

M13 phage coat proteins namely pIII, pVI, pVII, PVIII and pIX are attempted for the display of antibodies and peptides. Of which pVIII, a major coat protein, occurs in 3000 copies (Figure 1). Hence, it is normally being used to enhance detection signal. Further, pIII, a minor coat protein, comprises of 406 amino acids and displays only 3 to 5 copies (Figure 1). The folded globular proteins like immunoglobulins are displayed as fusion with pIII proteins as shown in Figure 1. An important feature to be considered in adopting phage display for the expression of fusion proteins is the loss of coat protein functionality, a major limitation in phage display technology. The hybrid phages with modified coat proteins are used to overcome the issue of loss of coat protein functionality. These hybrid virions comprise of a full set of wild type genome, a copy of fusion gene, origins of replication for phage and its host, gene 3 with cloning sites and antibiotic resistant gene. The wild pIII protein and fusion pIII protein are displayed on the surface of engineered phage. They are in the range of 1 to 9 and 1 to 1000 depending on the growth conditions.[20] The conceived antibody encodes a protease cleavage site between pIII and scFv fragment (single chain fragment variable) and hence the hyperphage-packed library can be eluted by protease treatment. The hybrid phage, helper phage and hyper phage[21] enable engineered phage to display large folded therapeutic proteins along with phage coat proteins as fusion proteins with least loss of coat protein functionality. Thus, made the phage display a well-designed tool with little economic implications and incompatibility issues compared to hybridoma technology. Intuitively, the expression of single chain variable fragments (scFv) attempted on M13 phage witnessed a ground-breaking discovery and consequently, the biopharma industries are revolutionizing their infrastructure related to the large-scale

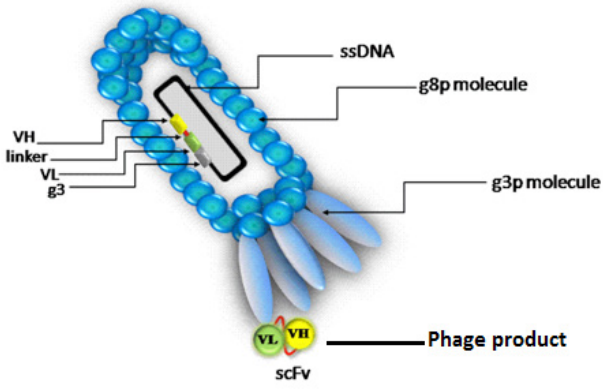

Figure 1: Schematic diagram showing the phage display of scFv. Source: www.kck.usm.my.[20]

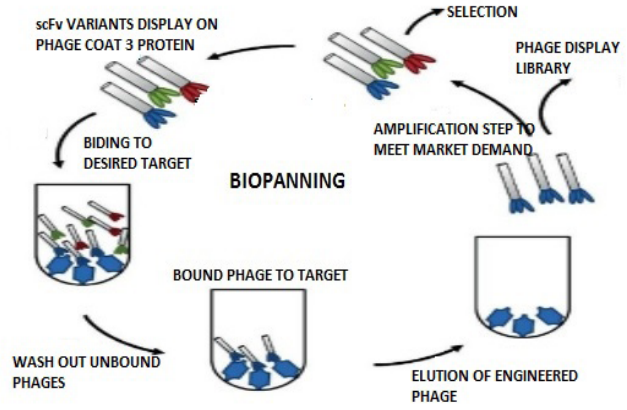

Figure 2: Schematic diagram showing the steps involved in the selection of engineered phages through biopanning.

production of recombinant antibody formats using M13 phage particles.[22]

Biopanning

The *in vitro* screening of antibodies from libraries is known as biopanning which is one of the prime steps in enhancing and harvesting phage displayed therapeutic proteins/peptides. There are incubation, screening, washing, selection, elution, identification and harvesting stages in biopanning procedure. Hence, this step is an essential one to enrich phage displayed molecules. As this is an *in vitro* screening and selection method for conceived displayed phage binder to the target so as to create antibody libraries, each one of the individual steps are standardized successfully using M13 phage such as experimenting with the choice of eluting reagents and enzymes to cleave the protease site engineered between the conceived antibody and pIII coat protein on the surface of the phage.[22] In the selection, the conceived molecule engineered in displaying phage is made to immobilize on a solid support similar to microtitre plate well used in ELISA (Figure 2) and successfully the antigen-specific antibodies could be isolated. The non-binding antibody-phage is removed by repeated washing and bound antibody phage will be isolated for its reamplification in *E. coli*. Ultimately, the aforementioned steps enable to harvest scFv antibodies binding to specific epitopes through phage ELISA. The source for conceived molecule is mRNA for V-region of scFv from an immunized animal or differentiated human B-cells. These scFvs are of low molecular weight antibody formats (28 kDa) and formulated as drug conjugates which could easily penetrate inside the target cells. This property of penetration into the target cells is a unique attribute of phage derived antibody formats viz., diabody, scFv, Fab and single domain antibody and the same is lacking in monoclonal antibodies. For the first time, Rajesh Kumar *et al.*[23] developed a cost effective phage display technique for the generation of anti V3-scFvs against V3 (third variable region) of Clade C HIV-I envelope so as to propose therapeutic cure for HIV-I seropositive patients. They selected phage clones displaying scFv through biopanning upon antigen (V3C and V3B) coated plates. Further, it was observed that there is cross reactive clones of V3C and V3B peptides during biopanning.[23] These scientists conducted human phase-1 trials, deciphered the protective effect of anti V3-scFvs against subtypes A, B and C HIV-1 viruses and thus observed the prevention of disease progression.[24]

Phage display derived products

A few biological pharma firms manufacturing the phage display derived therapeutic products are given in Table 1. More than 70 antibody-based products are currently in use for cellular imaging and therapy amounting to billions of USD. Incidentally, they constitute 53% of FDA approved biopharmaceuticals.[24]

Bactriophage in Pharma Market

There has been a resurgence of interest in bacteriophage across the globe for their immense role in food and clinical applications in the past 20 years. Their potential to be used as phage display systems, anti-bacterials, vehicles for vaccines and biocontrol agents had rekindled the attention in the corporate sector. The key players presently engaged in global bacteriophage market include Federal State Scientific Industrial Company Microgen, AmpliPhi Biosciences Corporation, Pherecydes Pharma, TechnoPhage SA, VersatileBio EnBiotix, Phage Biotech Ltd., Fixed-Phage Limited, InnoPhage, etc. Food and beverages also occupy the largest revenue share in the global bacteriophage market due to its widest applications in food biocontrol and green method

Table 1: A few phage display derived pharma products.

S.No.	Development name	Trade name	Target	Year of Approval/ status	Marketing company	Pathological Indications studied
1	Adalimumab (D2E7)	Humira	Tumor necrosis factor (TNF)	2002	AbbVie	Rheumatoid arthritis
2.	Belimumab	Benlysta	B-lymphocyte stimulator	2011	GSK	Systemic lupus erythematosus
3.	Necitumumab	Portrazza	Epidermal growth factor receptor	2015	ImClone/ Lilly	Squamous non-small cell lung cancer (NSCLC)
4.	Ramucirumab	Cyramza	Vascular endothelial growth factor receptor	2014	ImClone/ Eli Lilly	Gastric cancer, colorectal cancer and NSCLC
5.	Ranibizumab	Lucentis	Vascular endothelial growth factor A	2006	Genentech	Macular degeneration
6.	Raxibacumab	Abthrax	Protective antigen	2012	GSK	Anthrax
7.	Ecallantide	Kalbitor	Plasma kallikrein	2009	Dyax Corp.	Hereditary angioedema
8.	Ganitumab	AMG 479	Insulin-like growth factor receptor (IGF-1R)	Phase 2	Amgen	Cancer (pancreatic, colorectal breast, NSCLC)
9.	Tribananib	AMG386	Angiotensin 1 and 2 neutralizing peptibody	Phase 3	Amgen	Ovarian, fallopian tube and peritoneal cancers

that uses lytic bacteriophages derived from the environment to target pathogenic bacteria.[25] A rising significance of bacteriophages in the bacterial fermentation process also has led to growing interest of their role in manufacturing commodity chemicals, biotechnology products and food products such as ready-to-eat meals, fresh cut fruits, vegetables and dairy products. Increased use of bacteriophages in diagnostics, phage display technology and potential use in drug discovery and development are the main attributes to the fast growth of this segment.[25] In 2018, AmpliPhi Bioscience Corporation initiated clinical trials using phage therapy technique against *Pseudomonas aerogenosa* infection in cystic fibrosis.[25] The aforementioned companies are developing bacteriophage platforms and phagebanks for the treatment of multidrug resistance bacteria in emergency conditions. The global bacteriophage market size in the year 2017 was $567.9 Mn and an expected increase by 2026 would be $797.2 Mn with a predicted 3.9 % compound annual growth rate.[26]

CONCLUSION

The Noble Laureates Paul Berg (1980), George Kohler and Cesar Milstein (1984) and George P Smith and Sir Gregory P Winter (2018) contribution revolutionized the techniques in molecular biology and subsequently led to a simple technique namely Phage Display for the production of peptides and antibody formats compatible to the host systems. Further, M13 phage tolerates experimental conditions better than tailored B-cells and that gave an additional advantage to adapt phage in the development of therapeutic biological. Therefore, the global bacteriophage mediated pharm market is enhancing particularly in diagnostics, cellular imaging, antibody formats, drug discovery and development. These are the main attributes to the fast growth of phage display technology. These wide applications have led these filamentous bacteriophage strains to explore so as to enhance the global market potential for restoration of human health.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

rDNA: Recombinant deoxyribonucleic acid; **OKT3:** Monoclonal antibodies against CD3 containing T-lymphocytes; **CD:** Cluster of differentiation; **CH:** Constant Heavy region; **IgG:** Immunonoglobulin gamma; **F(ab):** Fragment antigen binding; **CDR:** Complementarity determining region; **CTLA-4:** Cytotoxic T lymphocyte

associated antigen-4; **APC:** Antigen presenting cell; **PD:** Programmed cell death protein; **Tc:** Cytotoxic T lymphocyte; **PD-L:** Ligand for PD; **scFv:** Single chain variable fragment; **ELISA:** Enzyme linked immunosorbent assay; **kDa:** Kilodaltons; **HIV:** Human immunodeficiency virus; **mRNA:** messenger ribonucleic acid; **V3C:** Variable 3 C Clade; **V3B** Variable 3 B Clade; **FDA:** Food and Drug Administration; **USD:** United states of America dollars; **Mn:** Million.

REFERENCES

1. Gringeri A. Factor VIII safety: Plasma-derived versus recombinant products. Blood Transfusion. 2011;9(4):366-70.
2. Tiede A, Brand B, Fischer R, Kavakli K, Lentz SR, Matsushita T, *et al*. Enhancing the pharmacokinetic properties of recombinant factor VIII: First-in-human trial of glyco PEG ylated recombinant factor VIII in patients with hemophilia A. Journal of Thrombosis and Haemostasis. 2013;11(4):670-8.
3. Milstein C. The hybridoma revolution: An offshoot of basic research. Bioessays. 1999;21(11):966-73.
4. Nieri P, Donadio E, Rossi S, Adinolfi B, Podestà A. Antibodies for therapeutic uses and the evolution of biotechniques. Curr Med Chem. 2009;16(6):753-79.
5. Guijarro HC. Monoclonal antibodies against PCSK9: From bench to clinic. Clin Investig Arterioscler. 2016;28(Suppl 2):14-21.
6. Frenzel A, Kügler J, Helmsing S, Meier D, Schirrmann T, Hust M, *et al*. Designing Human Antibodies by Phage Display. Transfus Med Hemother. 2017;44(5):312-8.
7. Fasslrinner F, Arndt C, Koristka S, Feldmann A, Altmann H, Bonin MV, *et al*. Midostaurin abrogates CD33-directed UniCAR and CD33-CD3 bispecific antibody therapy in acute myeloid leukaemia. Br J Haematol. 2019;186(5):735-40. doi: 10.1111/bjh.15975.
8. Emmons C, Hunsicker IG. Muromonab-CD3 (Orthoclone OKT3): The first monoclonal antibody approved for therapeutic use. Iowa Med. 1987;77(2):78-82.
9. Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, *et al*. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood. 1997;90(6):2188-95.
10. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, *et al*. Interleukin-2 receptor blockade with declizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. N Engl J Med. 1998;338(3):161-5.
11. Chen HX, Gore-Langton RE, Cheson BD. Clinical trials referral resource: Current clinical trials of the anti-VEGF monoclonal antibody bevacizumab. Oncology. 2001;15(8):1023-6.
12. Ecker DM, Jones SD, Levine HI. The therapeutic monoclonal antibody market. MAbs 2015;7(1):9-14.
13. Allison JP. 'Checkpoints'. Cell. 2015;162:1203-5.
14. Iwai Y, Shida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA. 2002;99(19):12293-7.
15. Smith GP, Petrenko VA. Phage Display. Chem Rev. 1997;97(2):391-410.
16. Luzi S, Kondo Y, Bernard E, Stadler LK, Vaysburd M, Winter G. Subunit disassembly and inhibition of TNFα by a semi-synthetic bicyclic peptide. Protein Eng Des Sel. 2015;28(2):45-52.
17. Nixon AE, Sexton DJ, Ladner RC. Drugs derived from phage display: From candidate identification to clinical practice. MAbs. 2014;6(1):73-85.
18. Ledsgaard L, Kilstrup M, Karatt-Vellatt A, McCafferty J, Laustsen AH. Basics of Antibody Phage Display Technology. Toxins. 2018;10(6):236. doi: 10.3390/toxins10060236.
19. Bazan J, Całkosiński I, Gamian A. Phage display--a powerful technique for immunotherapy: 1. Introduction and potential of therapeutic applications. Hum Vaccin Immunother. 2012;8(12):1817-28.
20. Susanne R, Joachim K, Frank B, Stefan D. A helper phage to improve single-chain antibody presentation in phage display. Nature Biotechnology. 2001;19(1):75-81.
21. Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman BM, Lee SM, *et al*. Single-chain antigen-binding proteins. Science. 1988;242(4877):423-6.
22. Siang TC, Yee SC, Zoltán K, Theam SL. Chapter 2.4. Generation of recombinant antibodies by display technologies for diagnostic applications. In Sustainable diagnostics for low resources areas, First Edition. Penerbit Universiti Sains Malaysia. 2017;1-15. ISBN 978-967-461-163-7, Pub.
23. Rajesh K, Hilal AP, Tripti S, Subrata S, Kalpana L. Phage display antibody libraries: A robust approach for generation of recombinant human monoclonal antibodies. International Journal of Biological Macromolecules. 2019;135:907-18.
24. Rajesh K, Ruchi K, Lubina K, Anurag S, Hilal AP, Ashutosh T, *et al*. Isolation and characterization of cross-neutralizing human anti-V3 single-chain variable fragments (scFvs) against HIV-1 from an antigen preselected phage library. Appl Biochem Biotech. 2019;187(3):1011-27.
25. www.credenceresearch.com/report/bacteriophage-market, Bacteriophage Market Size, Share, Trend, Growth and Forecast. 2018.
26. News item: Bacteriophage Market to Rear Excessive Growth during 2028 Versatile Bio, Pherecydes Pharma and Market Research Gazette. 2019.

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