Pel-Freez® BIOLOGICALS

WHITE PAPER

Searching For Complement Serum?

Technical Guide for Sourcing Complement Serum for Vaccine Immunogenicity and Efficacy Testing

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BACKGROUND

Vaccines are a fundamental weapon in our arsenal to provide effective protection against infectious diseases. Vaccines induce immune responses in the form of two types of antibodies: 1) those that bind pathogen antigens, but do not confer any functional immune responses, and 2) those that bind pathogen antigens and are functional in promoting the elimination of the pathogen or pathogen-infected cells to provide protection against infection or reduce disease severity [1].

Pel-Freez Biologicals has supplied complement serum throughout the development life cycle for many successful vaccine programs. This white paper is intended to help vaccine developers source the correct complement based on the type of vaccine and assay needed.

Functional antibodies may act alone or in conjunction with other host defense mechanisms, such as the complement system [2]. Quantitative tests such as the enzyme-linked immunosorbent assay (ELISA) measure immunogenicity of vaccines by detecting antibody binding to protective and nonprotective pathogen-specific epitopes; however, binding alone does not guarantee that the antibodies can effectively destroy the pathogen and protect the host from disease [3,4].

Functional immunoassays such as the opsonophagocytic killing assay (OPKA), the serum bactericidal assay (SBA) and neutralizing assays measure both immunogenicity and functional antibody immune responses. For certain pathogens, such as *Streptococcus pneumoniae* and *Neisseria spp.*, these functional immunoassays have been correlated with vaccine efficacy [1,5].

Primary sources of complement serum include healthy human blood donors, baby rabbits and guinea pigs [6,7]. Pel-Freez Biologicals has supplied Baby Rabbit Complement and Guinea Pig Complement to vaccine developers for many years. Recently, Pel-Freez Biologicals has developed Antibody Depleted Human Complement, which overcomes several of the common limitations of traditional non-human complement sources.

This white paper is intended to serve as a technical guide for vaccine developers and professionals tasked with sourcing complement sera for vaccine immunogenicity and efficacy testing and provide information for sourcing complement serum for a variety of vaccine development applications.

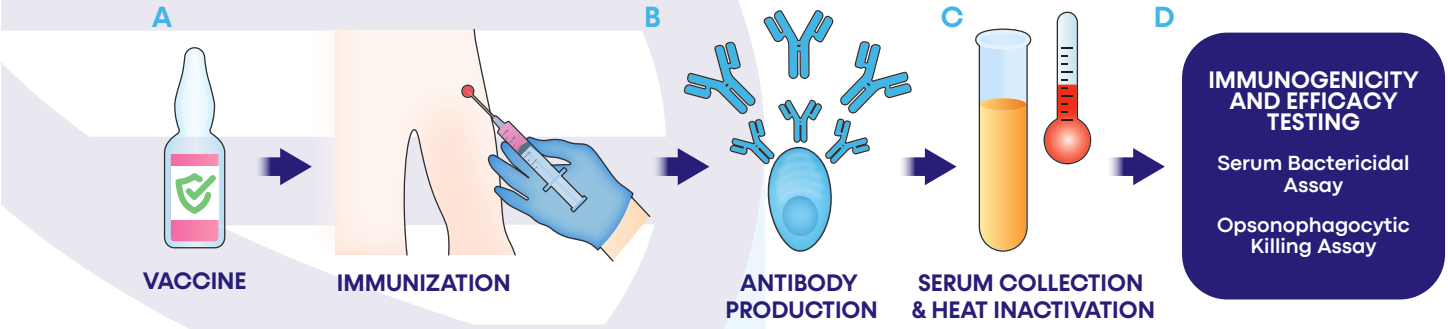


Figure 1. Strategy for vaccine immunogenicity and efficacy testing. **A)** Following vaccination, **B)** antibodies are produced specific for the pathogen targeted by the vaccine. **C)** The recipient's serum is collected for measurement of functional antibodies specific to the pathogen. Since the recipient's own complement proteins would destroy the target pathogen, the serum is heat-inactivated to destroy inherent, or endogenous, complement activity. **D)** The serum is then tested via the serum bactericidal or opsonophagocytic killing assay using a second, or exogenous, source of serum that retains complement activity.

COMPLEMENT USE DURING THE VACCINE DEVELOPMENT LIFE CYCLE

Prior to clinical vaccine studies in humans, small quantities of complement sera are needed (100 mL – 1 L) to establish SBA or OPKA protocols. Once Phase I clinical studies begin, the volume of complement serum required in testing will increase (1 L – 10 L) for vaccines that induce complement-dependent immune responses. As the number of vaccine tests increase in Phase II, complement serum usage will likely increase several-fold. Phase III clinical testing requires the largest volume of complement serum for the very large number of tests required. While complement serum requirements decrease following licensure, post-licensure monitoring studies will continue to demand substantial volumes of qualified complement. Other studies conducted to measure functional immunity induced by the vaccine throughout the life cycle such as vaccination in particular geographic areas, among particular age groups and/or the combination of the vaccine among these groups or with other vaccines will also require complement serum [8]. Qualification of replacement complement serum is absolutely critical for Phase III clinical testing and beyond and must be considered throughout vaccine development [9]. Primary sources of complement are human serum with interfering antibodies removed or immunologically-naïve 3-4 week rabbits.

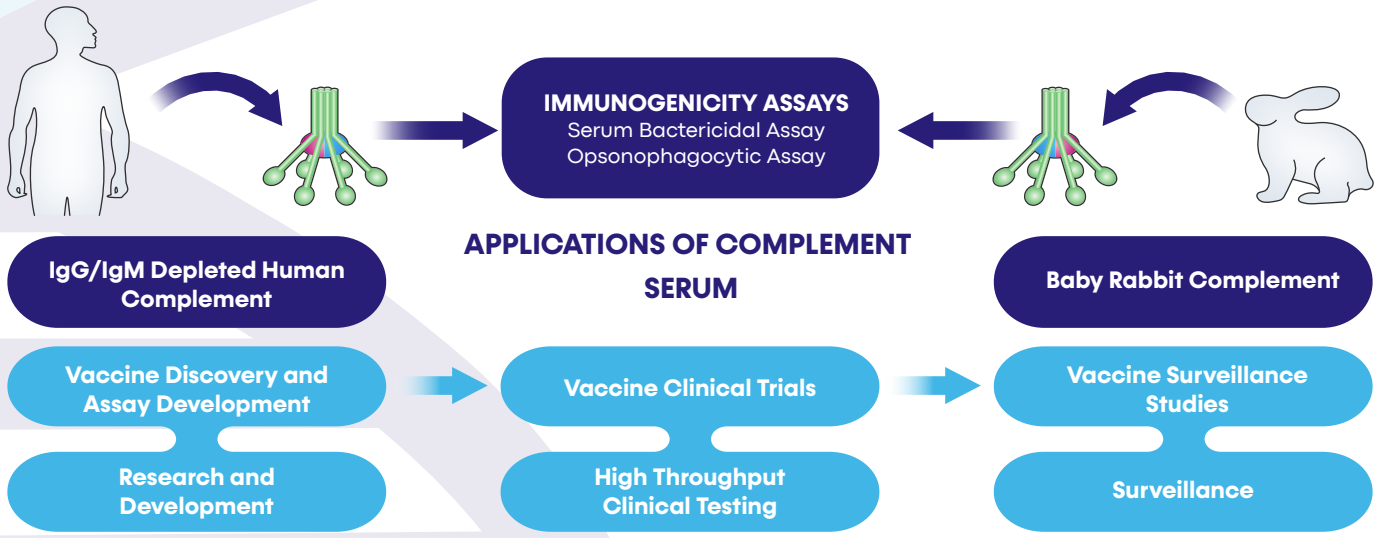


Figure 2. Immunogenicity and efficacy tests rely heavily on exogenous human and baby rabbit complement serum throughout the vaccine development life cycle.

Complement Usage in Vaccine Development

Stage or Phase	Complement Assays	Volume	Annual Estimates*	Immunogenicity Testing	Efficacy Testing	Safety Testing	Purpose
Exploratory	Possible	Negligible	0-100 mL	Possible	No	N/A	Identification of antigen(s), vector and type of vaccine
Pre-Clinical	Likely	Small	100 mL-1 L	Yes, if animal models are used	No	Tissue culture	To detect an immune response including animal protection if a suitable model is available
IND Application							
Phase I	Yes	Medium	1-10 L	Yes	No	Humans (20-100)	To determine safety, detection of immune response and its extent
Phase II	Yes	Large	4-40 L	Yes	No	Humans (400-1,000)	To assess safety, immunogenicity, proposed doses, schedule of immunizations and method of delivery
Phase III	Yes	Very Large	12-100 L	Yes	Yes	Humans (1,000-60,000)	To assess safety, immunogenicity and efficacy in a large group of people
Biologics License Application							
Post-Licensure Monitoring	Yes	Medium	1-10 L	Yes	Yes	Humans (>100,000)	To continue to assess vaccine safety, immunogenicity and efficacy

*Can vary depending on number of strains and/or antigens required and % complement used

Table 1. Complement usage throughout the vaccine development life cycle.

IMMUNOGENICITY ASSAY PRINCIPLE

Immunogenicity assays are used to measure functional antibodies elicited following vaccination. Figure 3 below provides a schematic diagram of the assay design: Step 1) Serum from a vaccine recipient is heat-inactivated to destroy endogenous complement activity, serially diluted, and then mixed with a constant amount of exogenous complement serum. Step 2) The mixture is combined with the target pathogen then plated on growth medium to observe pathogen survival in the presence of antibodies and complement. Step 3) Following incubation, the serum-complement-bacteria mixture is streaked onto agar plates and incubated again to visualize the reduction of colonies due to complement activation and subsequent lysis of bacteria. Step 4) Colonies are counted to determine the dilution that results in 50% reduction of bacteria.

Two of the most common sources of exogenous complement serum for clinical studies are immunologically-naïve rabbits and healthy humans. Regulatory agencies may insist on certain complement sources for certain target pathogens. As an example, the World Health Organization (WHO) has designated baby rabbit complement (BRC) as the preferred source of exogenous complement for opsonophagocytic killing assays (OPKA) for certain pathogens such as *Streptococcus pneumoniae* [10]. Antibody Depleted Human Complement is a viable alternative for applications for which human-derived complement is preferred, particularly for *Neisseria spp*. [11,12].

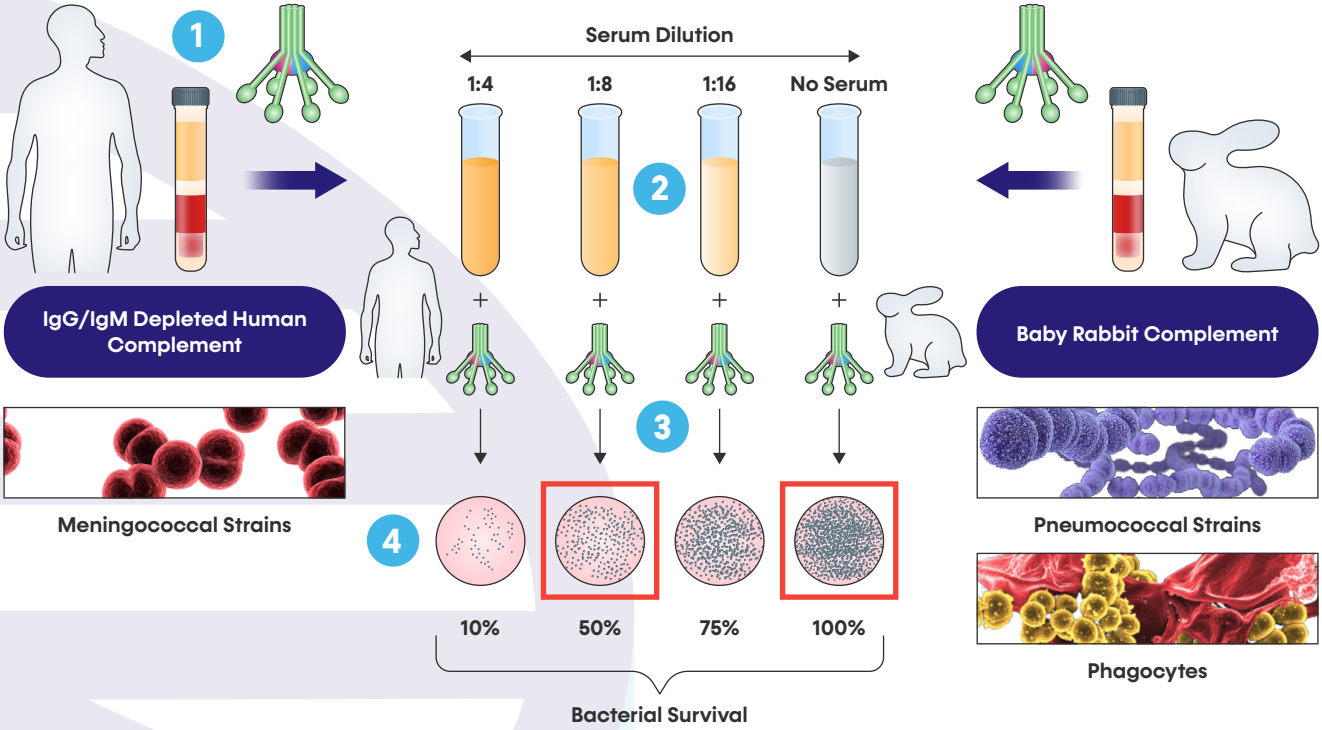


Figure 3. Principle of complement-based functional immunogenicity assays.

SERUM BACTERICIDAL ASSAY

The serum bactericidal assay is commonly used to test vaccine efficacy for Gram-negative bacteria species [13]. Gram-negative bacteria have an outer membrane and a thin peptidoglycan layer that is susceptible to direct lysis by complement proteins via the membrane attack complex (MAC).

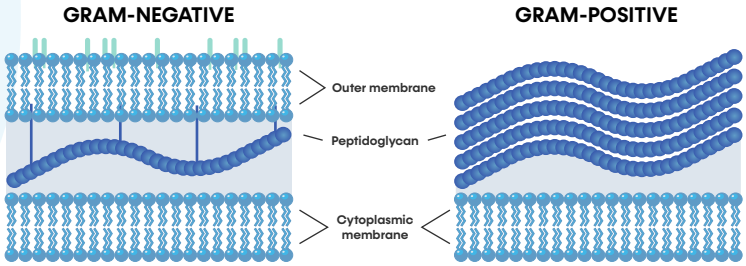
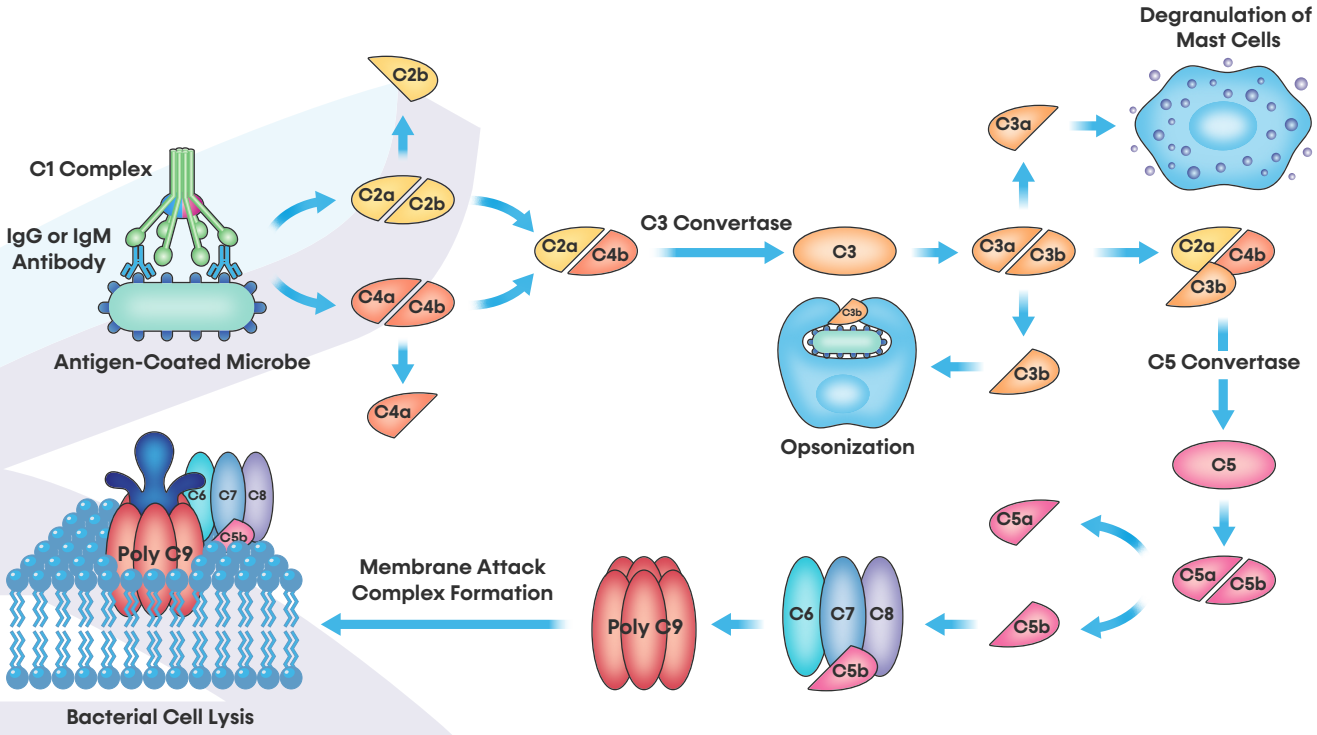


Figure 4. Bacterial cell wall and outer membrane structure determine susceptibility to complement-mediated lysis. **A**) Gram-negative species include *Escherichia coli*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*. **B**) Gram-positive species targets include *Staphylococcus aureas* and *Streptococcus pneumoniae*.

OPSONOPHAGOCYTIC KILLING ASSAY

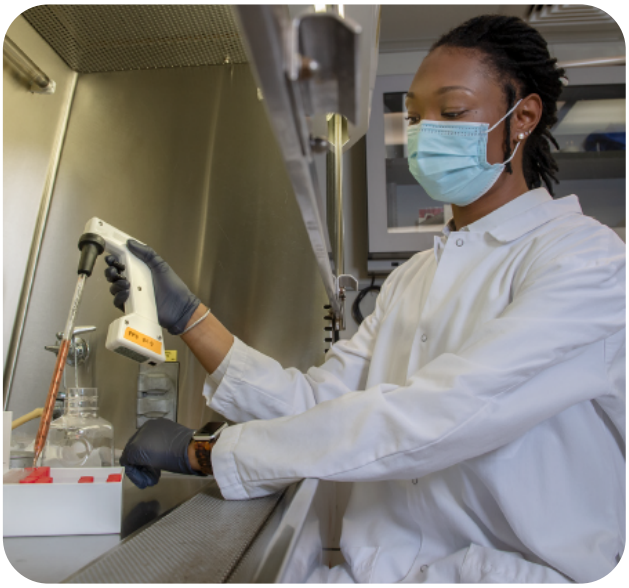
The opsonophagocytic killing assay is commonly used to test vaccine efficacy for Gram-positive bacteria species [14,15,16]. Gram-positive bacteria have a thicker protective peptidoglycan outer layer that is more resistant to direct lysis by the complement membrane attack complex as compared with Gram-negative bacterial species. Due to this resistance, bacterial lysis relies on the help of phagocytes that recognize complement proteins attached to IgG and IgM antibodies on the surface of the bacteria.

CLASSICAL COMPLEMENT PATHWAY

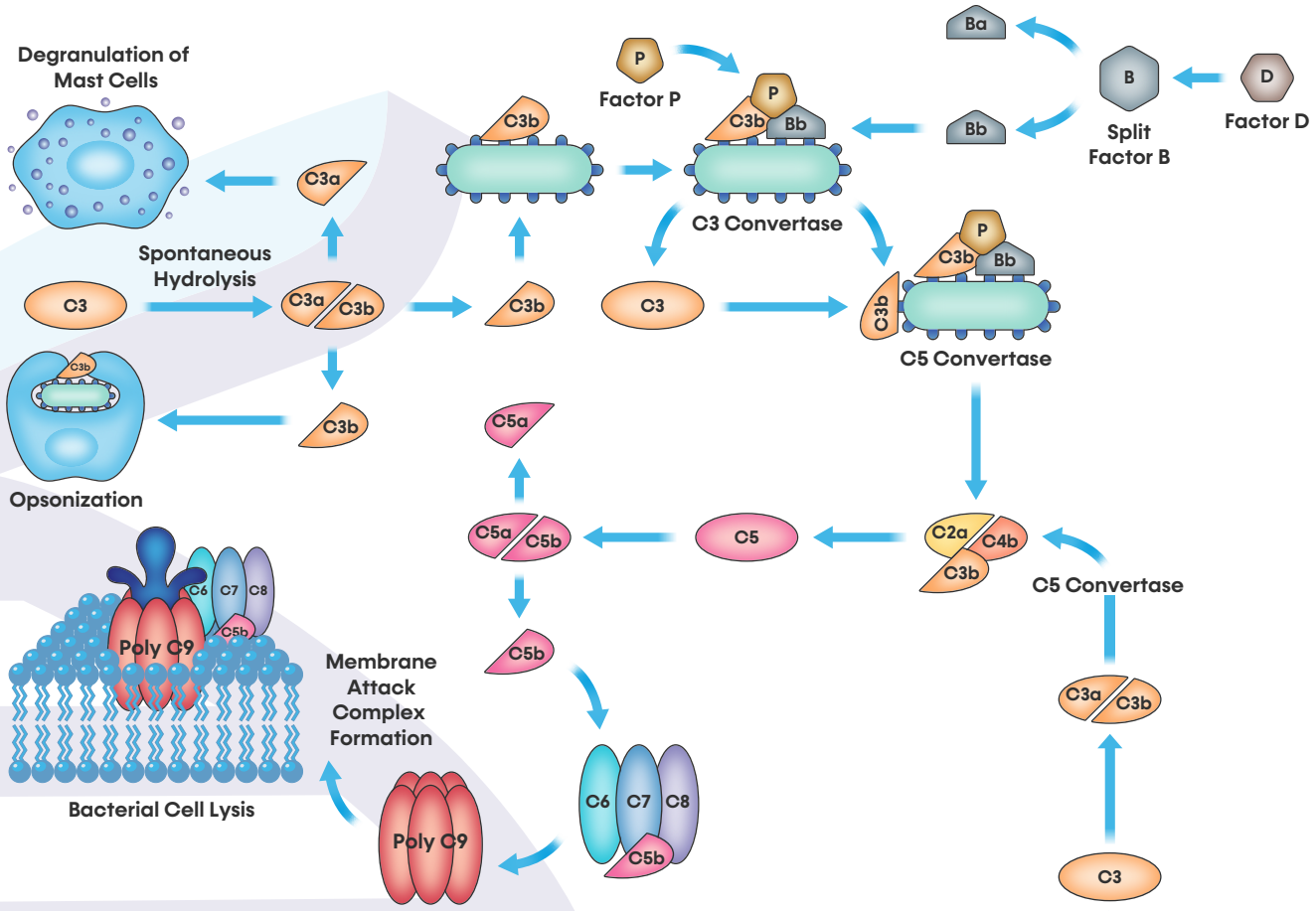


The Classical Complement Pathway begins with the binding of IgG or IgM antibodies to receptors on the surface of bacteria and other pathogens. Complement protein C1, a complex of C1q, C1r and C1s, recognizes several closely-spaced monomeric IgG molecules or single pentameric or hexameric IgM molecules following conformational changes in the Fc portion of these antibodies. The C1-antibody-antigen complex then triggers a cascade of proteolytic cleavage events that lead to the formation of C3 and C5 convertases. First, C2 and C4 bind to C1 forming the C3 convertase. Biologically active C3a and C3b fragments are formed following activation of the C3 convertase, each with numerous biological functions. C3a plays a role in T cell activation and survival, angiogenesis stimulation, chemotaxis, mast cell degranulation, and macrophage activation [17]. C3b is the most versatile of all complement proteins possessing the ability to complex with C3 convertase to form C5 convertase while also binding covalently to the target cell surface, serving as an opsonin by interacting with phagocyte receptors for target cell recognition and phagocytosis. Following activation of C5 convertase, C5 is cleaved into biologically active C5a and C5b fragments. C5a is a highly inflammatory peptide, attracting phagocytes and causing mast cells to release histamine. C5b complexes with C6, C7, C8 and C9 forming the MAC which inserts into the target cell membrane forming a transmembrane pore and osmotic channel disrupting the target cell membrane leading to cell lysis [18].

Complement serum is collected carefully from clotted whole blood after centrifugation and contains a family of active, heat-labile proteolytic proteins that make up the complement system. Through complement-dependent cytotoxicity (CDC) via the classical, alternative, and lectin pathways, complement proteins work with phagocytes and antibodies to destroy and clear pathogens from the body.



ALTERNATIVE COMPLEMENT PATHWAY



The Alternative Complement Pathway begins with spontaneous hydrolysis leading to cleavage of complement serum protein C3 into C3a and C3b subunits, followed by covalent binding of the C3b protease to the microbial cell surface. Serum Factor D cleaves serum Factor B into 2 fragments, Ba and Bb, followed by Bb binding to the microbial bound C3b to form C3bBb. This C3bBb is stabilized by a serum protein, Factor P, also known as properdin, to form C3bBbP. C3bBbP, which is still bound to the microbe, functions as a C3 convertase. Additional free C3 in the serum is cleaved by the C3 convertase and a second C3b is incorporated into the complex to form C3bBbPC3b which functions as a C5 convertase. C5 convertase then follows the classical pathway to form the MAC lysing the target cell [19,20].

LECTIN COMPLEMENT PATHWAY

The Lectin Complement Pathway is initiated by the binding of Mannose -binding lectin (MBL) or ficolin to pathogen surface receptors activating Mannose-binding lectin-Associated Serine Proteases (MASPs). While this pathway is independent of C1 and does not require antibody-antigen complexes, C3 and C5 convertases are generated upon activation leading to MAC formation and target cell lysis [21]. Certain bacteria such as *Salmonella, Listeria* and *Neisseria* species and some fungal pathogens such as *Candida albicans* and *Cryptococcus neoformans* activate the Lectin Pathway as well as viruses including HIV-1 and respiratory syncytial virus (RSV) [43].

ADDITIONAL COMPLEMENT OPTIONS

Guinea Pig Complement

Guinea Pig (GP) Complement has been used historically due to its 3 to 5-fold higher activity via the classical pathway as compared to human or rabbit complement serum and improved stability over less common complement sera such as mouse or rat [22].

Uses of Guinea Pig Complement include:

- *Vibrio cholerae* [37]
- *Salmonella minnesota* [36]
- Respiratory Syncitial Virus [43]
- Chandipura Virus [42]

Refer to Appendix A and B for additional uses of Guinea Pig Complement.

Keys to Complement Assay Success...

Include proper assay controls to confirm low intrinsic bactericidal activity:

- **Mix bacteria and complement in absence of antibody.**
- **Mix bacteria, antibody and heat-inactivated complement to confirm absence of interfering antibodies in the complement.**

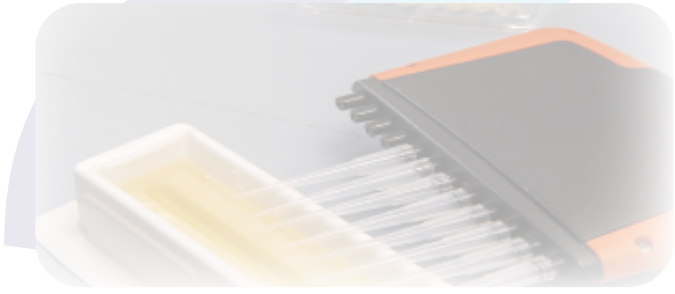
Other Uses of Complement

Active complement is also used in parasite-based studies f or the antibody-dependent lysis of trophozoites [54] and sporocytes [55].

Refer to Appendix B for additional details regarding the use of Complement in parasite-based assays.

"For over a decade Pel-Freez Biologicals has been a consistent supplier of high-quality reagents and an excellent partner."

Pfizer Vaccine Research & Development



Viral Uses of Complement

Viruses can be neutralized with complement using single or multiple complement pathways. The mechanism of viral neutralization includes virolysis of cell free virus, phagocytosis of C3b-coated viral particles, lysis of virus infected cells and aggregation of virions complexed with C1q, C3 and C4. Viral assay applications include:

- Micro neutralization assay [22]
- Single-radial hemolysis (SRH) repeatability assay [23]

Refer to Appendix B for additional uses of Complement in viral assays.

"Pel-Freez is our first choice as a supplier for biological matrices and complement. Their product quality and consistency of product lot-tot-lot enables us to develop premier bacterial assays in support of the worlds` premier vaccine biopharmaceutical companies with confidence."

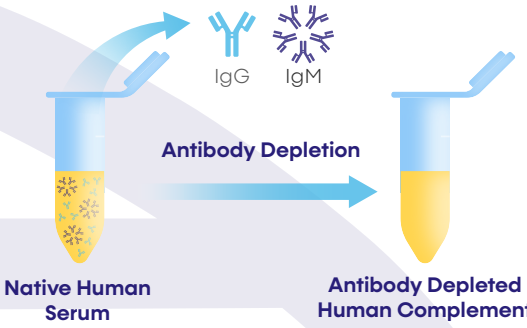
nexelis

PRODUCT INFORMATION SUMMARY: ANTIBODY DEPLETED HUMAN COMPLEMENT

Pel-Freez Biologicals now offers a large scale commercial source of human complement with residual antibodies removed for use in vaccine efficacy testing assays.

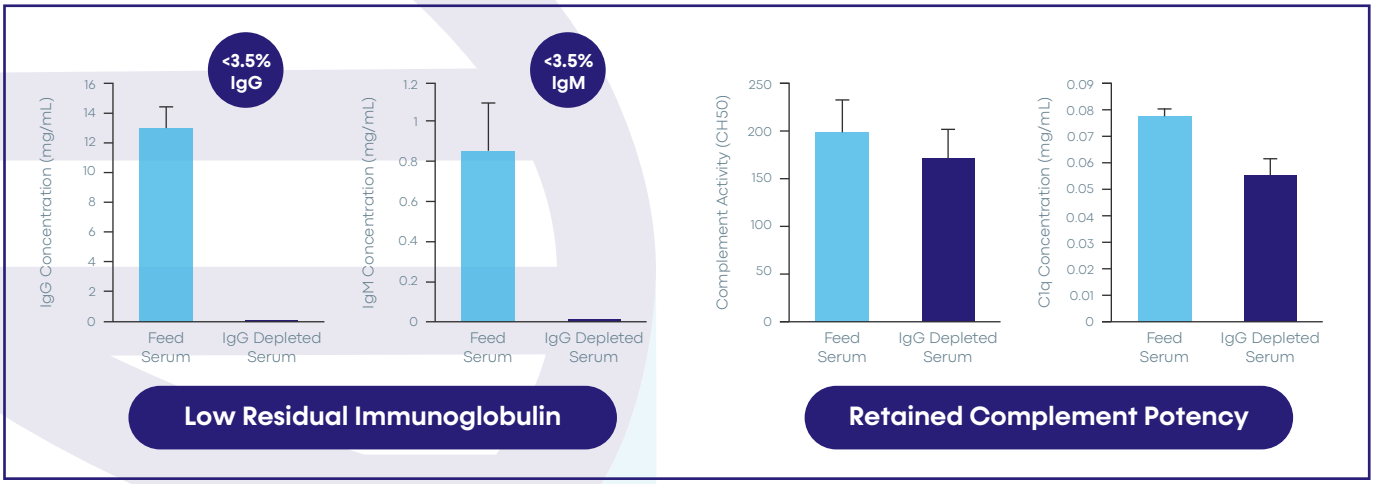
Prior to 2017 commercial sources of human complement with the necessary properties for vaccine immunogenicity testing and sufficient quantities for long-term clinical trials did not exist. While vaccine developers have established methods of removing interfering antibodies to produce viable human complement serum for their SBAs and OPKAs, these methods are tedious, consume valuable resources and require lengthy, ongoing trial-and-error testing [24].

Given our established role as a supplier of BRC, we were aware of the need for a commercial source for human complement that could be used in assays where human complement is preferred over BRC. Since the majority of human blood donors possess a variety of interfering antibodies to vaccine targets, these antibodies must be removed to have a viable source of human complement for vaccine immunogenicity tests. We have developed a method to remove the IgG and IgM antibodies that cause unwanted intrinsic killing while maintaining sufficient complement potency. Beginning with small lots of a few hundred milliliters, we found that the product worked in a variety of applications and we successfully scaled our process to 1 L+ lots in late 2018. Now our antibody depleted human complement is available for use in vaccine efficacy programs in volumes sufficient to support clinical trials for many years.



The Antibody Depletion Advantage

- **Improved Lot-to-Lot Consistency**
- **Reduced Screening Time**
- **Liter+ Quantities Available**



"Pel-Freez's IgG/IgM depleted human complement has been crucial for complement-dependent bactericidal assays in my lab. The material is of the highest quality, with minimal lot-to-lot variation. This reagent has saved us considerable time and expense. Pel-Freez's human complement will be invaluable for any lab or company developing vaccines that rely on complement activation for efficacy."

Dr. Sanjay Ram
Professor

Human serum is processed from whole blood acquired from healthy donors in FDA-licensed facilities in the U.S. Donors provide informed consent prior to donation. Each unit of blood is tested according to FDA guidelines and found to be negative for all required viral markers.

PRODUCT INFORMATION SUMMARY: ANTIBODY DEPLETED HUMAN COMPLEMENT

Manufacturing Process Overview

IgG and IgM immunoglobulins are depleted from pooled normal human serum using proprietary methods. Antibody depleted complement is 0.2 micron filtered then filled in a variety of pack sizes from 1 mL to 100 mL. Quality control assays include residual IgG and IgM content and complement activity via hemolytic titer (CH50). See Figure 5 below for an overview of the general manufacturing process.

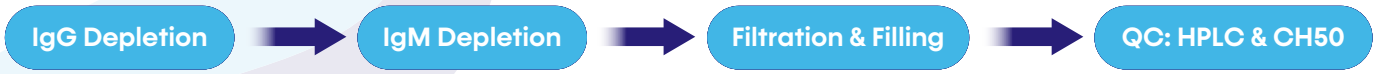


Figure 5. Flow chart for the manufacture of antibody depleted human complement.

Product	Product Code	Aliquot Size	Specifications
IgG/IgM Depleted Human Complement Pooled Serum	31010-1	1 mL	Hemolytic: Titer ≥ 1:100 Residual IgG & IgM: <3.5% each vs. feed serum Typical Lot Size: 1 L to 2.5 L
	31010-2	2 mL	
	31010-5	5 mL	
	31010-10	10 mL	
	31010-100	100 mL	

Table 2. Pel-Freez Biologicals Antibody Depleted Human Complement available pack sizes and specifications.

"Pel-Freez is our go-to provider of essential reagents such as Lyophilized Rabbit complement and immunoglobulin-depleted human serum. These quality products allow us to perform our in-house opsonophagocytic killing assay (OPK/OPA) and move rapidly on product development. We have ordered from Pel-Freez for over a decade and they are always willing to go above and beyond in making sure we can obtain the right product for our specific research needs."

IMMUNE DIAGNOSTICS

Dr. Joseph E. Martinez
President, Immune Diagnostics
Former Vaccine Scientist, CDC

Since 2017, Pel-Freez Biologicals has supplied large volumes of human complement to vaccine developers for multi-strain assays using both the serum bactericidal and opsonophagocytic killing formats. This commercial source of human complement has enabled vaccine developers worldwide to reallocate their time and resources previously spent on making small quantities of human complement to new vaccine discoveries.

"Human complement is an essential reagent for much of our research and product testing. We have prepared our own IgG- and IgM-depleted complement from donor blood for more than 20 years and would not consider using a commercial product unless it showed consistent antibody depletion and complement activity. After rigorous testing, we were extremely pleased to find the Pel-Freez depleted human complement to meet all our needs. This has saved us a considerable amount of time and expense allowing us to focus on experiments instead of preparing reagents."

OMVax, Inc.

Dr. Gregory R. Moe
Chief Scientific Officer
OMVax, Inc.

BULK PRICING AVAILABLE

APPENDIX A. Examples of Complement Assays Performed with Bacterial Pathogens

Gram-Negative Bacteria Applications				
Organism	**Antibody Source**	**Assay Type**	**Complement Source**	**Ref.**
Borrelia burgdorferi	Murine	Growth Inhibition Assay	Guinea Pig	[25]
Chlamydia pneumoniae	Properdin (as a Pattern Recognition Molecule)	C3b Deposition & Intracellular Immunostaining of EB	Human	[26]
Escherichia coli	Human	SBA	Human	[27]
Escherichia coli	Human	OPA	Human, BRC	[28]
Escherichia coli	Human	Viability Staining & Bioluminescence	Human	[29]
Enteroaggregative *Escherichia coli*	Human	C3b Deposition and Bacterial Killing	Human	[30]
Haemophilus influenzae type b	Human	SBA	BRC, Goats, Horses, Bovine Calves, Pigs and 8-12 week old Rabbits	[31]
Klebsiella pneumoniae	Human	Bacterial Luminescence C3 Opsonization	Human	[32]
Neisseria gonorrhoeae	Human	Phagocytic Killing & C3 Deposition	Human	[33]
Neisseria meningitidis	Human	SBA	Human, BRC	[34]
Salmonella enterica serovars	Murine	SBA	BRC, Goats, Horses, Bovine Calves, Pigs and 8 -12 week old Rabbits	[35]
Salmonella enterica serovar Typhimurium	Human	SBA	Human	[36]
Vibrio cholera	Human	Vibriocidal Antibody Assays	Guinea Pig	[37]
Gram-Positive Bacteria Applications				
Bacillus subtillis	Human	Viabilty Staining & Bioluminescence	Human	[29]
Staphylococcus aureus	Human	OPA	BRC	[38]
Streptococcus agaiactiae (GBS)	Human or Rabbit	OPA	BRC	[39]
Streptococcus pneumoniae	Human	OPA	BRC	[40]
Streptococcus pyogenes (GAS)	Human or Rabbit	OPA	BRC	[41]

APPENDIX B. Examples of Complement Assays Performed with Viral and Parasitic Pathogens

Viral Applications			
Organism	**Antibody Source**	**Complement Source**	**Ref.**
Chandipura Virus	C1q	Human	[42]
Respiratory Syncytial Virus	Human	Guinea Pig	[43]
Zika Virus	C1q	BRC	[44]
Vesicular Stomatitis Virus	Human	Human	[45]
Human Immunodeficiency Virus	Human	Human	[46]
West Nile Virus	Murine	BRC	[47]
Sindbis Virus	Murine	Human	[48]
Epstein-Barr Virus	Human	Human	[49]
Mumps Virus	CV-1 cells	Guinea Pig	[50]
Influenza Virus	BHK-21 cells	Guinea Pig	[51]
Herpes Simplex Virus 2	Human	Human	[52]
Hepatitis C Virus	Murine and Rabbit	Human	[53]
Parasite Applications			
Giardia lamblia	IgM (but not IgG) from Symptomatic Giardiasis Patients	Human, Guinea Pig	[54]
Plasmodium falciparum	Sporozoite-specific IgG & IgM Human Antibodies	Human	[55]

References

1. Plotkin, S. 2010. Correlates of protection induced by vaccination. Clini Vaccine Immunol. 17:1055-1065. https://doi.org/10.1128/CVI.00131-10

2. Goldschneider I, Gotschlich EC, Artenstein MS. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med. 129:1307-26. https://doi.org/10.1084/jem.129.6.1307 PMID: 4977280. PMCID: PMC2138650

3. Gill CJ, Ram S, Welsch JA, Detora L, Anemona A. 2011. Correlation between serum bactericidal activity against *Neisseria meningitidis* serogroups A, C, W-135 and Y measured using human versus rabbit serum as the complement source. Vaccine. 30:29-34. https://doi.org/10.1016/j.vaccine.2011.10.068

4. Borrow R, Carlone GM, Rosenstein N, Blake M, Feavers I, Martin D, Zollinger W, Robbins J, Aaberge I, Granoff DM, Miller E, Plikaytis B, van Alphen L, Poolman J, Rappuoli R, Danzig L, Hackell J, Danve B, Caulfield M, Lambert S, Stephens D. *Neisseria meningitidis* group B correlates of protection and assay standardization--international meeting report Emory University, Atlanta, Georgia, United States, 16-17 March 2005. Vaccine. 2006 Jun 24:5093-107. https://doi.org/10.1016/j.vaccine.2006.03.091 PMID: 16838413.

5. Bash M, Lynn F, Mocca B, Borrow R, Findlow H, Hassan-King M, Preziosi MP, Idoko O, Sow S, Kulkarni P, LaForce FM. 2014. Development and use of a serum bactericidal assay using pooled human complement to assess responses to a meningococcal group A conjugate vaccine in African toddlers. Clin Vaccine Immunol. 21:755-761. https://doi.org/10.1128/CVI.00812-13

6. Zollinger WD, Mandrell RE. 1983. Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. Infection and Immunity. 40:257-264. https://doi.org/10.1128/iai.40.1.257-264.1983 PMCID: PMC264843

7. Santos GF, Deck RR, Donnelly J, Blackwelder W, Granoff DM. 2001. Importance of complement source in measuring meningococcal bactericidal titers. Clin Diag Lab Immunol. 8:616-623. https://doi.org/10.1128/CDLI.8.3.616-623.2001 PMCID: PMC96111

8. Martin D, McCallum L, Glennie A, Ruijne N, Blatchford P, O'Hallahan J, Oster P. 2005. Validation of the serum bactericidal assay for measurement of functional antibodies against group B meningococci associated with vaccine trials. Vaccine. 23:2218-2221. https://doi.org/10.1016/j.vaccine.2005.01.070 PMID: 15755599

9. Jackson LA, Baxter R, Reisinger K, Karsten A, Shah J, Bedell L, Dull PM, V59P13 Study Group. 2009. Phase III comparison of an investigational quadrivalent meningococcal conjugate vaccine with the licensed meningococcal ACWY conjugate vaccine in adolescents. Clin Infect Diseases. 49:e1-10. https://doi.org/10.1086/599117

10. Recommendations for the production and control of pneumococcal conjugate vaccines. In: WHO Expert Committee on Biological Standardization. Fifty-fourth report. Geneva, World Health Organization, 2005, Annex 2 (WHO Technical Report Series, No. 927). https://cdn.who.int

11. Beernink PT, Ispasanie E, Lewis LA, Ram S, Moe GR, Granoff DM. 2019. A meningococcal native outer membrane vesicle vaccine with attenuated endotoxin and overexpressed factor H binding protein elicits gonococcal bactericidal antibodies. J Infect Dis. 219:1130-1137. https://doi.org/10.1093/infdis/jiy609 PMID: 30346576. PMCID: PMC6420169.

12. Gulati S, Pennington MW, Czerwinski A, Carter D, Zheng B, Nowak NA, DeOliveira RB, Shaughnessy J, Reed GW, Ram S, Rice PA. 2019. Preclinical efficacy of a lipooligosaccharide peptide mimic candidate gonococcal vaccine. mBio. 10:e02552-19. https://doi.org/10.1128/mBio.02552-19 PMID: 31690678. PMCID: PMC6831779.

13. McIntosh EDG, Broker M, Wassil J, Welsch JA, Borrow R. 2015. Serum bactericidal antibody assays – the role of complement in infection and immunity. Vaccine. 33:4414-4421. https://doi.org/10.1016/j.vaccine.2015.07.019

14. Baltimore RS, Kasper DL, Baker CJ, Goroff DK. 1977. Antigenic specificity of opsonophagocytic antibodies in rabbit anti-sera to group B streptococci. J Immunol. 8: 673-8. https://pubmed.ncbi.nlm.nih.gov/65433/ PMID: 65433.

15. Romero-Steiner S, Frasch C, Concepcion N, Goldblatt D, Käyhty H, Väkeväinen M, Laferriere C, Wauters D, Nahm MH, Schinsky MF, Plikaytis BD, Carlone GM. 2003. Multi-laboratory evaluation of a viability assay for measurement of opsonophagocytic antibodies specific to the capsular polysaccharides of *Streptococcus pneumoniae*. Clin Diagn Lab Immunol. 10:1019-24. https://doi.org/10.1128/cdli.10.6.1019-1024.2003 PMID: 14607861. PMCID: PMC262452.

16. Burton RL, Nahm MH. 2006. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. Clin Vaccine Immunol. 13:1004-9. https://doi.org/10.1128/CVI.00112-06 PMID: 16960111. PMCID: PMC1563573.

17. da Silva WD, Eisele JW, Lepow IH. 1967. Complement as a mediator of inflammation. 3. Purification of the activity with anaphylatoxin properties generated by interaction of the first four components of complement and its identification as a cleavage product of C'3. J Exp Med. 126:1027-48. https://doi.org/10.1084/jem.126.6.1027 PMID: 6069927. PMCID: PMC2138425.

18. Kolb WP, Haxby JA, Arroyave CM, Müller-Eberhard HJ. 1972. Molecular analysis of the membrane attack mechanism of complement. J Exp Med. 135(3):549-66. https://doi.org/10.1084/jem.135.3.549 PMID: 5058233. PMCID: PMC2139138.

19. Koethe SM, Austen KF, Gigli I. 1973. Blocking of the hemolytic expression of the classical complement sequence by products of complement activation via the alternate pathway. Materials responsible for the blocking phenomenon and their proposed mechanism of action. J Immunol. 110:390-400. https://www.jimmunol.org/content/110/2/390.long PMID: 4684033.

20. May JE, Frank MM. 1973. A new complement-mediated cytolytic mechanism - the C1-bypass activation pathway. PNAS. 70:649-52. https://doi.org/10.1073/pnas.70.3.649 PMID: 4123687; PMCID: PMC433326.

21. Müller E, Schröder C, Schauer R, Sharon N. 1983. Binding and phagocytosis of sialidase-treated rat erythrocytes by a mechanism independent of opsonins. Hoppe Seylers Z Physiol Chem. 364:1419-29. https://doi.org/10.1515/bchm2.1983.364.2.1419 PMID: 6642429.

22. Abai AM, Smith LR, Wloch MK. 2007. Novel microneutralization assay for HCMV using automated data collection and analysis. J Immunol Methods. 322:82-93. https://doi.org/10.1016/j.jim.2007.02.001 PMID: 17343873. PMCID: PMC1933494.

23. Fulton RE, DiNinno VL, Frank RI, Fildes J, Turner IJ. 1984. Single radial hemolysis test for quantitation of complement-fixing antibodies to non-hemagglutinating viruses. J Clin Microbiol. 20:248-54. https://doi.org/10.1128/jcm.20.2.248-254.1984 PMID: 6386847. PMCID: PMC271297.

24. Brookes C, Kuisma E, Alexander F, Allen L, Tipton T, Ram S, Gorringe A, Taylor S. 2013. Development of a large-scale human complement source for use in bacterial immunoassays. J Immunol Methods. 391:39-49. https://doi.org/10.1016/j.jim.2013.02.007 PMID: 23485926.

25. Comstedt P, Hanner M, Schüler W, Meinke A, Lundberg U. 2014. Design and development of a novel vaccine for protection against lyme borreliosis. PLoS ONE. 9:e113294. https://doi.org/10.1371/journal.pone.0113294

26. Cortes C, Ferreira VP, Pangburn MK. 2011. Native properdin binds to *Chlamydia pneumoniae* and promotes complement activation. Infect Immun. 79:724-31. https://doi.org/10.1128/IAI.00980-10

27. Mutti M, Ramoni K, Nagy G, Nagy E, Szijártó V. 2018. A new tool for complement research: *In vitro* reconstituted human classical complement pathway. Front Immunol. 9:2770. https://doi.org/10.3389/fimmu.2018.02770

28. Abbanat D, Davies TA, Amsler K, Hea W, Fae K, Janssen S, Poolman JT, van den Dobbelsteen GPJM. 2017. Development and qualification of an opsonophagocytic killing assay to assess immunogenicity of a bioconjugated *Escherichia coli* vaccine. Clin Vaccine Immunol. 24:e00123-17. https://doi.org/10.1128/cvi.00123-17.

29. Virta M, Lineri S, Kankaanpää P, Karp M, Peltonen K, Nuutila J, Lilius EM. 1998. Determination of complement-mediated killing of bacteria by viability staining and bioluminescence. Appl Environ Microbiol. 64:515-9. https://doi.org/10.1128/AEM.64.2.515-519.1998

30. Adler Sørensen C, Rosbjerg A, Hebbelstrup Jensen B, Krogfelt KA, Garred P. 2018. The lectin complement pathway is involved in protection against enteroaggregative *Escherichia coli* infection. Front Immunol. 9:1153. https://doi.org/10.3389/fimmu.2018.01153

31. Kim HW, Kim KH, Kim J, Nahm M. 2016. A high throughput serum bactericidal assay for antibodies to *Haemophilus influenzae* type b. BMC Infect Dis. 16:473. https://doi.org/10.1186/s12879-016-1808-4

32. Nypaver CM, Thornton MM, Yin SM, Bracho DO, Nelson PW, Jones AE, Bortz DM, Younger JG. 2010. Dynamics of human complement–mediated killing of *Klebsiella pneumoniae*. Am J Respir Cell Mol Biol. 43:585–590. https://doi.org/10.1165/rcmb.2009-0292OC

33. Ross SC, Densen P. 1985. Opsonophagocytosis of *Neisseria gonorrhoeae*: Interaction of local and disseminated isolates with complement and neutrophils. J Infect Disease. 151:33-41. https://doi.org/10.1093/infdis/151.1.33

34. Findlow J, Balmer P, Borrow R. 2019. A review of complement sources used in serum bactericidal assays for evaluating immune responses to meningococcal ACWY conjugate vaccines. Human Vaccines & Immunotherapeutics. 15:2491-2500. https://doi.org/10.1080/21645515.2019.1593082

35. Boyd MA, Tennant SM, Saague VA, Simon R, Muhsen K, Ramachandran G, Cross AS, Galen JE, Pasetti MF, Levine MM. 2014. Serum bactericidal assays to evaluate typhoidal and nontyphoidal Salmonella vaccines. Clin Vaccine Immunol. 21:712-721. https://doi.org/10.1128/CVI.00115-14

36. O'Shaughnessy CM, Cunningham AF, MacLennan CA. 2012. The stability of complement-mediated bactericidal activity in human serum against Salmonella. PLoS ONE. 7: e49147. https://doi.org/10.1371/journal.pone.0049147

37. Charles RC, Hilaire IJ, Mayo-Smith LM, Teng JE, Jerome JG, Franke MF, et al. 2014. Immunogenicity of a killed bivalent (O1 and O139) whole cell oral cholera vaccine, Shanchol, in Haiti. PLoS Negl Trop Dis 8:e2828. https://doi.org/10.1371/journal.pntd.0002828

38. Nanra JS, Buitrago SM, Crawford S, Ng J, Fink PS, Hawkins J, Scully IL, McNeil LK, Aste-Amézaga JM, Cooper D, Jansen KU, Anderson AS. 2013. Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Human Vaccines & Immunotherapeutics. 9:480-487. https://doi.org/10.4161/hv.23223

39. Guttormsen HK, Liu Y, Paoletti LC. 2008. Functional activity of antisera to group B streptococcal conjugate vaccines measured with an opsonophagocytosis assay and HL-60 effector cells. Human Vaccines. 4:370-374. https://doi.org/10.4161/hv.4.5.5988

40. Romero-Steiner S, Libutti D, Pais LB, Dykes J, Anderson P, Whitin JC, Keyserling HL, Carlone GM. 1997. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. Clin Diagn Lab Immunol. 4:415-22. https://doi.org/10.1128/cdli.4.4.415-422.1997 PMID: 9220157. PMCID: PMC170543.

41. Salehi S, Hohn CM, Penfound, TA, Dale JB. 2018. Development of an opsonophagocytic killing assay using HL-60 cells for detection of functional antibodies against *Streptococcus pyogenes*. mSphere. 3: e00617-18. https://doi.org/10.1128/mSphere.00617-18

42. Kunnakkadan U, Nag J, Kumar NA, Mukesh RK, Suma SM, Johnson JB. 2019. Complement-mediated neutralization of a potent neurotropic human pathogen, Chandipura virus, is dependent on C1q. Journal of Virology. 93:e00994-19. https://doi.org/10.1128/JVI.00994-19

43. Fuentes S, Crim RL, Beeler J, Teng MN, Golding H, Khurana S. 2013. Development of a simple, rapid, sensitive, high-throughput luciferase reporter based microneutralization test for measurement of virus neutralizing antibodies following Respiratory Syncytial Virus vaccination and infection. Vaccine. 31:3987-3994. https://doi.org/10.1016/j.vaccine.2013.05.088

44. Schiela B, Bernklau S, Malekshahi Z, Deutschmann D, Koske I, Banki Z, Thielens NM, Würzner R, Speth C, Weiss G, Stiasny K, Steinmann E, Stoiber H. 2018. Active human complement reduces the Zika virus load via formation of the membrane-attack complex. Front Immunol. 9:2177. https://doi.org/10.3389/fimmu.2018.02177

45. Beebe DP, Cooper NR. 1981. Neutralization of Vesicular Stomatitis Virus (VSV) by human complement requires a natural IgM antibody present in human serum. J Immunol. 126:1562-1568. https://pubmed.ncbi.nlm.nih.gov/6259260/ PMID: 6259260.

46. Aasa-Chapman MMI, Holuigue S, Aubin K, Wong M, Jones NA, Cornforth D, Pellegrino P, Newton P, Williams I, Borrow P, McKnight A. 2005. Detection of antibody-dependent complement-mediated inactivation of both autologous and heterologous virus in primary Human Immunodeficiency Virus Type 1 infection. J Virology. 79:2823-2830. https://doi.org/10.1128%2FJVI.79.5.2823-2830.2005

47. Mehlhop E, Whitby K, Oliphant T, Marri A, Engle M, Diamond MS. 2005. Complement activation is required for induction of a protective antibody response against West Nile Virus infection. J Virology. 79:7466-7477. https://doi.org/10.1128%2FJVI.79.12.7466-7477.2005

48. Hirsch RL, Griffin DE, Winkelstein JA. 1983. Natural immunity to Sindbis Virus is influenced by host tissue sialic acid content. PNAS. 80:548-550. https://doi.org/10.1073/pnas.80.2.548

49. Sairenji T, Sullivan JL, Humphreys RE. 1984. Complement-dependent, Epstein-Barr Virus-neutralizing antibody appearing early in the sera of patients with infectious mononucleosis. J Infect Disease. 149:763-768. https://doi.org/10.1093/infdis/149.5.763

50. Hirsch RL, Wolinsky JS, Winkelstein JA. 1986. Activation of the alternative complement pathway by mumps infected cells: Relationship to viral neuraminidase activity. Arch Virology. 87:181-190. https://doi.org/10.1007/bf01315298

51. Reading PC, Hartley CA, Ezekowitz RAB, Anders EM. 1995. A serum mannose-binding lectin mediates complement-dependent lysis of Influenza Virus-infected cells. Biochem Biophys Res Comm. 217:1128-1136. https://doi.org/10.1006/bbrc.1995.2886.

52. Crisci E, Ellegard R, Nystrom S, Rondahl E, Serrander L, Bergstrom T, Sjowall C, Eriksson K, Larsson M. 2016. Complement opsonization promotes Herpes Simplex Virus 2 infection of human dendritic cells. J Virology. 90:4939-4950. https://doi.org/10.1128/JVI.00224-16

53. Brown KS, Keogh MJ, Owsianka AM, Adair R, Patel AH, Arnold JN, Ball JK, Sim RB, Tarr AW, Hickling TP. 2010. Specific interaction of Hepatitis C Virus glycoproteins with mannan binding lectin inhibits virus entry. Protein Cell. 1:664-674. https://doi.org/10.1007/s13238-010-0088-9

54. Deguchi M, Gillin FD, Gigli I. 1987. Mechanism of killing of *Giardia lamblia* trophozoites by complement. J Clin Invest. 79:1296-1302. https://doi.org/10.1172/JCI112952

55. Behet MC, Kurtovic L, van Gemert GJ, Haukes CM, Siebelink-Stoter R, Graumans W, van de Vegte-Bolmer MG, Scholzen A, Langereis JD, Diavatopoulos DA, Beeson JG, Sauerwein RW. 2018. The complement system contributes to functional antibody-mediated responses induced by immunization with *Plasmodium falciparum* malaria sporozoites. Infection and Immunity. 86:e00920-17. https://doi.org/10.1128/IAI.00920-17

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