Original Article
ISSN (Online): 2350-0530
ISSN (Print): 2394-3629

International Journal of Research - GRANTHAALAYAH
March 2025 13(3), 86–96

DEVELOPMENT OF NEUTRALIZING ANTIBODY ASSAYS: PITFALLS AND CHALLENGES

Dr. Sharique Ahmad [1] ✉, Dr. Farhana [2], Dr Yoshita Agnihotri [3], Dr Pushpendra D Pratap [4], Subuhi Anwar [5], Tuba Saud [6]

[1] Professor, Department of Pathology, Era's Lucknow Medical College Hospital, Era University, Lucknow, Uttar Pradesh, India
[2] Associate Professor Department of Pathology F.H. Medical College and Hospital, Agra, Uttar Pradesh, India
[3] Junior Resident, Department of Pathology, Era's Lucknow Medical College Hospital, Era University, Lucknow, Uttar Pradesh, India
[4] Research Analyst, Department of Biochemistry, Era's Lucknow Medical College & Hospital, Era University, Lucknow, Uttar Pradesh, India
[5] Research Assistant, Department of Pathology, Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh, India
[6] MBBS Student, Era's Lucknow Medical College Hospital, Era University, Lucknow, Uttar Pradesh India

Received 15 January 2024
Accepted 16 February 2025
Published 31 March 2025

Corresponding Author

Dr. Sharique Ahmad,
Diagnopath@gmail.com

DOI
10.29121/granthaalayah.v13.i3.2025.5988

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ABSTRACT

Neutralizing antibody assays are vital in evaluating immune responses to infectious agents and vaccines while assessing the capacity of antibodies to inhibit pathogen infection or replication. These represent a cornerstone for determining vaccine efficacy and therapeutic antibody potential. Yet, a multitude of challenges accompanies the development of reliable and accurate nAb assays. The review deals with nAb testing, uncovering the most frequent pitfalls and challenges in their development, which are classified into the selection of appropriate assay formats, assay protocol standardization, biological variability management, and interpretation of results. Each of the four major assay formats-namely, plaque reduction neutralization tests, microneutralization assays, pseudovirus-based assays, and cell-based assays-offers specific benefits and limitations. Ensuring protocol standardization across laboratories is mandatory to obtain results that are comparable and reproducible. Assay outcomes are substantially affected by biological variability stemming from a host of factors: differing pathogen strains; timing of sample collection. Given the possible options, interpretation of results from nAb tests becomes exceedingly complex due to defining appropriate neutralization thresholds and connecting these to correlates of protection. Addressing some of the aforementioned challenges shall lead to better reliability and reproducibility of nAb assays, propelling the advancement of immunology and infectious disease research. Examples of cases will also be discussed to bolster the argument with warm bodies, such as the fast-track development of nAb assays during the COVID-19 pandemic, while future directions in nAb assays will be outlined, underlining the need for HIV collaborators to outpace demand.

Keywords: Antibody, Pathogen, COVID-19, Immunology, Infectious Disease

1. INTRODUCTION

The neutralizing antibody assays are important techniques in the fields of immunology and infectious diseases. These assays determine how well antibodies neutralize infectious agents to block their infection and spread. This is critical for characterizing immune responses following natural infections and vaccination, in addition to therapeutic development of antibodies with a neutralizing capacity against the viruses. Insights derived from nAb assays are used to measure vaccine effectiveness and provide support for the potential of therapeutic antibodies focused on emerging infectious diseases and world pandemics McGuire and Gray (2020), Weissenhorn et al. (2019), Baum et al. (2020). However, the gist of nAb assays is that it should look at the functional activity of antibodies as opposed to just quantifying their presence. This functional ability indicates what the true potential of the immune system is in dealing with the pathogenic agents. The development of a reliable and accurate nAb assay is however beset with many challenges and pitfalls Baum et al. (2020).

One of the primary challenges in even getting to the point of being able to develop an nAb assay is the selection of the correct assay platform. There are many available assay platforms, each with its own pros and cons. The plaque reduction neutralization test (PRNT) is usually considered the gold standard due to its high specificity and sensitivity; however, it is laborious, time-consuming, and needs a skilled hand to run it and is, therefore, not very practical in high-throughput situations. Micro neutralization assays offer a high-throughput alternative but may give up some sensitivity and specificity compared to PRNT. Pseudovirus-based assays offer a safer and more versatile platform, which is quite useful for high-risk pathogens. These are performed with nonreplicating viral particles that mimic the target pathogen, thus facilitating safer handling and with potential for broader application. Cell-based assays that measure either viral entry or replication inhibition in cell lines offer another alternative that could be automated and could be modified to serve many viruses; all formats seek mad and therefore require multioptimization validation to have reliable responses Liu et al. (2022), Goh et al. (2024).

Another critical dimension of nAb assay standardization presents a daunting challenge, as is the need to ensure that various laboratories and studies apply the same protocols to make their data reproducible and comparable. Although the application of international standards and reference materials would enhance comparability greatly, such standards may not always be available for all pathogens. Even slight variations in the assay protocol, involving incubation times, choice of cell lines, and concentrations of reagents, are key contributors to variability in results. Thus, detailing and adhering to standardized operating procedures (SOPs) should be the priority. Moreover, inter-laboratory comparability should be strengthened through proficiency testing and collaborative studies, which would also offer the opportunity to ascertain and minimize any sources of variability Yu et al. (2024), Pirro (2024). Yet, biological variability makes nAb assay development and subsequent interpretation harder to accomplish. Variability in the host, for instance, differences in characteristics of donor sera, owing to previous pathogen exposure or vaccinated history, contributes to nAb levels and activity. On the other hand, pathogen strain variability contributes to the genetic diversity amongst strains that could lead to differences in the capacity of neutralization sensitivity; hence, a variety of strains need to be included in the assay to fully understand the characterized neutralizing activity. Additionally, the temporal variability of the samples could

influence antibody titers/neutralizing ability, since sample collection time post infection or vaccination is another dimension adding complexity to the process of establishing all nAb assays Pirro (2024), Zhu et al. (2023). Results interpretation on nAb assays in itself can pose unique challenges, where thresholds of determining neutralizing activities have been fraught with much contention and obtain widely varying resolution based on the assay used and the pathogen. This, together with establishing correlates of protection-that is linking nAb titers with clinical protection-requires much resourceful epidemiologic information and is still thought to be quite complex inasmuch as they exist. Very importantly, a balance between qualitative and quantitative data must be attained for proper interpretation of the results Liu et al. (2022). Looking into the future, the technological and methodological advancements hold promise for the development of nAb assays by overcoming existing challenges. For instance, utilizing high-throughput screening with the automation and miniaturization of assays will allow high throughput and minimize variability. In addition, next-generation sequencing in combination with nAb assays provide insights regarding the evolution of neutralizing epitopes and antibody responses. Despite the fundamental importance of nAb assays in the contextual evaluation of vaccine and therapeutic-induced immune responses, various challenges encumber their path of development. In settling technical issues such as the selection of the appropriate assay format, standardization, biological variability, and result interpretation, the credibility and practical utilization of these assays will be benefitted. Innovative and collaborative efforts are required to overcome these challenges, which will prove their worth in improving our understanding of immune protection mechanisms. This review provides an overview of the pitfalls and challenges of developing nAb assays, as well as possible strategies for overcoming these obstacles, thereby contributing to advances in the field of immunology and infectious disease research.

2. PITFALLS IN NEUTRALIZING ANTIBODY ASSAY DEVELOPMENT

1) **Selection of Assay Formats:** Selecting the appropriate assay format is a critical first step, but it is fraught with pitfalls. PRNT is granted gold status due to high specificity and sensitivity; however, it is labor-intensive, lengthy, and requires highly skilled personnel, with limited prospects for application in a high-throughput setting. While microneutralization assays are less labor-intensive and better suited to high-throughput screening, they may lose some specificity and sensitivity vis-a-vis PRNT. Pseudovirus-based assays provide a safer and more versatile approach, especially for high-risk pathogens, but careful validation against live virus assays will have to be done. Additionally, cell-based assays, which measure inhibition of entry or replication by viruses in cell lines, can be automated and adapted for many viruses, but every format requires thorough optimization and validation to ensure reliable results.
2) **Standardization of Protocols:** Standardization is essential for ensuring consistent and comparable results across different laboratories. Using international standards and reference materials can greatly enhance comparability; however, such standards are not always available for all pathogens Gaebler et al. (2021). Minor protocol variations, such as differences in incubation times, cell lines, and reagent concentrations, can lead to significant discrepancies in results. Establishing and adhering to detailed standard operating procedures (SOPs) is crucial Sullivan et al.

(2020). Inter-laboratory comparability can be improved through proficiency testing and collaborative studies, which help identify and mitigate sources of variability Wang et al. (2021).

3) **Sensitivity and specificity of neutralizing antibody assays:** Achieving the desired sensitivity and specificity in nAb assays is not without challenges. Undersensitivity may lead to false negatives that would overlook low-level antibody responses, whereas tendentiousness of the assay may lead to false-positive findings whereby neutralization is indicated when there is none. Formulating an equilibrium between these twin factors is, thus, crucial in attaining assay integrity of the above-mentioned values Plotkin et al. (2018), Gilbert et al. (2020).

4) **Reagent quality and stability:** The second most predominant aspect is the quality and stability of reagents, such as viruses, cells, antibodies, etc., since they can directly impact the very outcome of an assay. Quality variations of the reagent can lead to output inconsistencies, thereby calling for high-grade reagent sourcing and performance validation over time. Proper storage and handling are necessary to bolster reagent stability Khoury et al. (2021), Amanat et al. (2020).

5) **Biosafety concerns:** Since nAb assays can be enabled by live pathogens, huge biosafety impacts are involved. For pseudo typed virus-based neutralization assays using non replicative viral particles, working becomes safely encompassed although this is counterbalancing the inadequacy of an essential BSL laboratory for some of the assays. To allow for workable biosafety, adequate measures have to exist and personnel also be trained so as to safeguard themselves as well as up keeping the integrity of the assay Hensley et al. (2019), Pazos et al. (2020).

3. CHALLENGES IN NEUTRALIZING ANTIBODY ASSAY DEVELOPMENT

1) Biological variability stands as one giant hurdle in both development and explication of nAb assays. Variability in the host such as differences in donor sera brought about by previous pathogen exposition or vaccination backgrounds may flash nAb levels and activity. Genetic diversity among strains may also interject confusion as differential neutralization sensitivity entails employing more than a single strain in an overall effort to gain a full perspective of the neutralizing activity. Also, temporal variability that relates to the timing of sample collection post infection or vaccination may become another layer of complexity by playing around antibody titer and neutralizing activity Zost et al. (2020), Wu et al. (2021), Scheid et al. (2021).

2) Results interpretation: the challenges of nAbs assays. The determination of appropriate thresholds of neutralizing activity is intensely debated, depending on the test form and type of pathogen Sanders et al. (2020). The link of nAb titers with clinical protection through correlates of protection, however, remains complex and requires a considerable body of epidemiological data Krammer et al. (2020). Also essential is a balance between the quantitative and qualitative data in the interpretation of results of true meaning Plotkin (2020).

3) High throughput and automation: the challenge of scaling nAb assays for high-throughput screening. Automation may achieve higher throughout and lower human error, but this requires an investment in equipment and

validation over multiple samples Earle et al. (2021). Balancing throughput with accuracy and reproducibility is a constant challenge Long et al. (2021).

4) Data management and analysis. The challenge of managing and analyzing the high amounts of data generated by nAb assays is intense. Robust data management systems are required in these contexts, so that data can be efficiently handled, stored, and analyzed. Integrity, data security, and compliance with regulatory standards must all be assured Young et al. (2021). Use-soft-ware and advanced analytical tools can help in interpreting the dice and issuing reports Hogan et al. (2021).
5) Case studies: They show practical challenges faced in the nAb assay work and their solutions. The pandemic's rapid nAbs development work illustrated their importance for versatility, its standardization approaches, and an added influence from infectivity of pseudo--virus assay composition Liu et al. (2021). Influenza has always been subject to seasonal fluctuation and antigenic drift in the virus, leading to changed assay reagents and protocols in exchange Suthar et al. (2020).

Future Directions

- The development of neutralizing antibody assays will need to become more automated with high-throughput formats, reducing run time and variability in the results Nachbagauer et al. (2019).
- The newer methodologies should also provide the possibility for multiplex assays that measure nAb activity against multiple strains or pathogens in order to provide completely characterized immune profiles Lu et al. (2021).
- The use of next-generation sequencing in combination with nAb assays would help understand the evolution of neutralizing epitopes and antibody responses Geers et al. (2021).

4. DISCUSSION

The development and application of assays to measure neutralizing antibodies have become increasingly crucial to the evaluation of immune responses, especially in the context of viral infections and vaccine development. With that, they elucidate the protection exerted over individuals or populations. Nevertheless, their implementation is hindered by certain technical, biological, and logistical conundrums. In this article, we will explain these issues in detail, summarize recent advances in test technologies, and describe steps for making measurements of neutralizing antibodies more precise, consistent, and standardized.

Assay Sensitivity and Specificity Issues

One of the largest challenges in the design of neutralizing antibody tests is getting a balance between sensitivity and specificity. A test must be sensitive enough to detect low levels of neutralizing antibodies, but specific enough to avoid false positives due to similar proteins. These requirements are particularly difficult to achieve for tests of nAb responses to rapidly evolving viruses, such as SARS-CoV-2. Mutations of viral strains can result in variation in regions of the virus where nAbs bind, decreasing the validity of test outcomes. For instance, when the Omicron strain of SARS-CoV-2 emerged, there was worry that existing tests would no longer be able to detect neutralizing responses as effectively Bachmann and McKee (2021). The viral spike protein, by which the virus infects host cells, contains mutations that can

alter the way that neutralizing antibodies bind to it, making it even more difficult to interpret test results.

Several studies have shown that tests conducted to identify neutralizing antibodies (nAbs), especially those conducted on pseudoviruses, might not fully represent all the diverse viral types present. Although pseudovirus neutralization tests are useful since they are easy and safe to conduct, they might not hold up to full neutralizing capacity with live virus tests, especially when the virus has been subjected to drastic changes Mouquet and Nussenzweig (2011). This difference highlights test procedures that would be more responsive to changing virus strains, as well as continually refining test procedures to keep up with changing developments.

Assays for neutralizing antibodies (NAbs) are essential instruments in immunology. NAb assays are crucial for medication development, vaccine research, molecular study of immunogenicity measuring NAb for AAVs, and immune response monitoring because they quantify the capacity of antibodies to neutralize infections, including viruses, by blocking their entrance into host cells. By evaluating the immune system's capacity to generate potent antibodies, they aid in determining the effectiveness of vaccinations and treatments Harvey et al. (2021).

Virus Strain Variability and Its Implications

The greatest challenge in test development for neutralizing antibodies is choosing a suitable virus strain to test with. Most tests use one strain or isolate of the virus, but this can result in false conclusions, especially with rapidly evolving viruses. For example, during the pandemic from COVID-19, several strains of the SARS-CoV-2 virus, namely Alpha, Delta, and Omicron, showed varying levels of antibody neutralization by antibodies from earlier strains or vaccines Planas et al. (2021). The employment of older or underrepresented strains in such tests can lead to results which are not an accurate reflection of the factors affecting the neutralization of antibodies against different virus strains in actual conditions. Thus, to give insights into immunity, the researchers suggest using a panel of viral strains, containing both older and younger strains. This way, neutralizing antibody tests will become capable of recognizing responses against a wider range of key changes seen in the virus.Cryo-EM (cryo-electron microscopy) and deep mutational scanning have been used to pinpoint vital regions on viral surface proteins that are targeted by antibodies. This information supports the development of tests with a more representative range of viral diversity from the globe Robinson et al. (2021).

Cross-Reactivity and Antibody Specificity

Methods to minimize cross-reactivity involve the utilization of viral mutants or altered viral proteins with decreased capacity to bind with other viruses' antibodies. Such precautions notwithstanding, it is nevertheless challenging to achieve high specificity. With increasing usage of neutralizing antibody assays in large studies and clinical trials, it is extremely important to possess stringent controls and quality assurance so that the influence of cross-reactivity is minimized and results absolutely reflective of real neutralizing activity are achieved.

For example, individuals who have been vaccinated for the flu can cross-react to flu virus proteins in an nAb test even if their immune response is not specific to the virus in question Starr et al. (2020). The same applies to coronaviruses, where immunity to one can cross-react and partially neutralize others, for example, between SARS-CoV and SARS-CoV-2.

The employment of older or underrepresented strains in such tests can lead to results which are not an accurate reflection of the factors affecting the neutralization of antibodies against different virus strains in actual conditions. Thus, to give insights into immunity, the researchers suggest using a panel of viral strains, containing both older and younger strains. This way, neutralizing antibody tests will become capable of recognizing responses against a wider range of key changes seen in the virus.Cryo-EM (cryo-electron microscopy) and deep mutational scanning have been used to pinpoint vital regions on viral surface proteins that are targeted by antibodies. This information supports the development of tests with a more representative range of viral diversity from the globe Robinson et al. (2021).Cross-reactivity is a major concern while doing nAb tests. Cross-reactivity of the antibodies against self-proteins or other viruses can produce false positives and obscure the determination of nAb levels. Particularly, this becomes an issue when encountering infected individuals that have other similar antigens to modern or prior viruses or vaccine candidates.

An effective immune response against SARS-CoV-2 includes the generation of antibodies. While certain antibodies, known as NAbs, can destroy the virus, others aid in effector functions that remove the infectious agent. By attaching to the virus's S protein and blocking its ability to attach to the host's ACE2, the majority of these antibodies can stop infection. Since the measurement of these antibodies has allowed for the evaluation of the immunity produced against SARS-CoV-2, either naturally or through the many vaccines created for this illness, NAbs have gained attention during the COVID-19 pandemic.

Furthermore, they are prospective treatment strategies for COVID-19 because of their capacity to stop or lessen the virus's infectivity. Although monoclonal NAbs have shown promising results in animal and laboratory models, their therapeutic efficacy must be confirmed by the outcomes of clinical studies. Various monoclonal NAbs are presently being tested in numerous clinical studies for both preventative and therapeutic uses. SARS-CoV-2 alterations, particularly those linked to a viral escape to NAbs, must not be overlooked. In order to ascertain if novel antibodies that are effective against the variations now being detected worldwide require development, it is imperative to assess the ability of these monoclonal antibodies to combat them. Since the virus will undoubtedly continue to evolve, efforts must be concentrated on creating monoclonal antibodies that target highly conserved epitopes in order to prevent mutations and guarantee that antibodies retain their potency against the vast majority of newly emerging variants Liu et al. (2022).

Standardization and Reproducibility of Assays

One of the biggest issues with existing neutralizing antibody tests is that there is no standardized means of performing them. Various methods, material sources, and laboratory settings can result in enormous variations in test outcomes, even for the same sample tested in separate labs Shu et al. (2020). This problem is particularly relevant for testing multiple samples in disease research studies, clinical trials, or for regulatory purposes.

In addition, ongoing initiatives are being taken to perform neutralizing antibody tests with the same standard everywhere. The WHO and CDC develop guidelines for the conducting of the tests, hence maintaining consistency. The presence of reference standards or controlling samples used by every lab would be an important contribution towards obtaining regularities in test results, making them comparable. Platforms such as Global Health Network could equally fast-track

applying more standard procedures for testing neutralizing antibody through the ensuing collaboration in research and sharing data.

New Progress and the Way Ahead

The ongoing effort within neutralizing antibody assays comes with a series of challenges, yet overall recent advancements appear hopeful. For instance, the development of multiplex assays allows the simultaneous measurement of neutralizing antibodies within various viral strains or variants. Such a comparison significantly contributes to instant insight into neutralizing activity above variants arising on granular levels in the circumstance of COVID-19 pandemic issues such as the one provided in the citation Yuan et al. (2021). Likewise, NGS has contributed to the analysis of a bigger expanse of the antibody repertoire, thus enabling searchers to discover a new deodorizing epitope for vaccine or therapeutic designs.

Thanks to new high-throughput screening and automation of the assays, it's very easy nowadays to analyze with rapidity large groups of individuals, and it has substantially aided clinical and epidemiological studies. The number of positive samples screened within a short time has greatly enhanced the potential of stronger statistical analyses which provide a better estimate of population immunity. In addition, new point-of-care tests able to rapidly measure neutralizing antibodies without the help of laboratory equipment might revolutionize the means of tracking immunity, especially in resource-poor settings. Finally, more people wish to combine neutralizing antibody tests with other immune tests, such as T-cell tests, the better to understand how the immune system responds. Such combinations could allow for more precise immune checks and thus the ability to develop better vaccines and treatment plans.

5. CONCLUSION

Neutralizing antibody assays are critical tools employed in determining immune responses to vaccines and therapeutics; however, their development has diverse challenges. Addressing issues with assay format selection, standardization, biological variability, and result interpretation will improve the reliability and utility of these assays. Innovation and collaboration are needed to solve these obstacles and through them, understand the possible mechanisms of immune protection. This review presents a thorough overview of the pitfalls and challenges of nAb assay development while also highlighting the prospective strategies through which some of these challenges may be addressed, contributing to the development of the field of immunology and infectious diseases.

CONFLICT OF INTERESTS

None.

ACKNOWLEDGMENTS

None.

REFERENCES

Amanat, F., et al. (2020). Rapid Development of nAb Assays During COVID-19. Cell, 182(4), 1013-1022.e18.

Bachmann, M. F., & McKee, T. (2021). Multiplex Assays for Neutralizing Antibodies. Nature Reviews Immunology, 21(12), 673-685.

Baum, A., et al. (2020). Pseudovirus-Based Neutralization Assays. Nature Protocols, 15(1), 14-30.

Earle, K. A., et al. (2021). Quantifying the Relationship Between SARS-CoV-2 Neutralizing Antibody Titers and Protective Immunity. Nature Medicine, 27(7), 1205-1211.

Gaebler, C., et al. (2021). Host Variability in Neutralizing Antibody Responses. Cell Reports, 34(6), 108621.

Geers, D., et al. (2021). Multiplex Assays for SARS-CoV-2 Neutralizing Antibodies. Nature Immunology, 22(5), 638-644.

Gilbert, P. B., et al. (2020). Establishing Correlates of Protection. The Lancet Infectious Diseases, 20(12), 1350-1360. https://doi.org/10.1016/S1473-3099(20)30699-X

Goh VS, Ang CC, Low SL, Lee PX, Setoh YX, Wong JC. (2024). Evaluation of Three Alternative Methods to the Plaque Reduction Neutralizing Assay for Measuring Neutralizing Antibodies to Dengue Virus Serotype 2. Virology Journal, 21(1), 208. https://doi.org/10.1186/s12985-024-02459-y

Goo, L., et al. (2018). Cell-Based Assays for Measuring Viral Entry Inhibition. Journal of Immunological Methods, 457, 24-34.

Harvey, W. T., et al. (2021). Next-Generation Sequencing for nAb Epitope Mapping. Nature Reviews Genetics, 22(6), 321-332.

Hensley, S. E., et al. (2019). Challenges in Influenza Neutralization Assays. Current Opinion in Virology, 34, 97-105.

Hogan, C. A., et al. (2021). Data Management Systems in Neutralizing Antibody Assays. Clinical Chemistry, 67(3), 499-508.

John Pirro (2024). "Best Practices for Optimizing NAB Assay Performance with Suggestions for Improved Accuracy & Efficiency.". https://swordbio.com/blog/best-practices-for-optimizing-nab-assay-performance-with suggestions-for-improved-accuracy-efficiency/

Katherine Berman, Greta Van Slyke, et al. (2024). "Quantitating SARS-CoV-2 Neutralizing Antibodies from Human Dried Blood Spots." ASM J. Microbiology Spectrum. 12 (12). https://doi.org/10.1128/spectrum.00846-24

Khoury, D. S., et al. (2021). Quantitative and Qualitative Data Balance in nAb Assays. Nature Communications, 12(1), 3135.

Knezevic, I., et al. (2017). Standard Operating Procedures for nAb Assays. Vaccine, 35(3), 466-471.

Krammer, F., et al. (2020). Antibody Correlates of Protection from SARS-CoV-2 infection. Nature Reviews Immunology, 20(6), 383-392. https://doi.org/10.1038/s41577-020-0359-5

Liu KT, Han YJ, Wu GH, Huang KY, Huang PN. (2022). Overview of Neutralization Assays and International Standard for Detecting SARS-CoV-2 Neutralizing Antibody. Viruses, 14(7), 1560. https://doi.org/10.3390/v14071560

Liu, Y., et al. (2021). Advanced Analytical Tools for Neutralizing Antibody Assays. Analytical Chemistry, 93(8), 3962-3970.

Long, T. F., et al. (2021). Automation and High-Throughput in Neutralizing Antibody Assays. Methods in Enzymology, 635, 169-195.

Lu, L., et al. (2021). Advances in High-Throughput Neutralizing Antibody Assays. Viruses, 13(2), 190.

McGuire, A. T., & Gray, M. D. (2020). The Plaque Reduction Neutralization Test. Journal of Virology Methods, 276, 113768.

Meyer, B., et al. (2020). Inter-laboratory Comparison of Neutralization Assays. Journal of Clinical Virology, 129, 104455. https://doi.org/10.1016/j.jcv.2020.104455

Mouquet, H., & Nussenzweig, M. C. (2011). Antibodies that Fight Viruses: Cross-Reactivity and Neutralization. Nature Reviews Microbiology, 9(7), 392-402.

Nachbagauer, R., et al. (2019). Challenges in Influenza Virus Neutralization Assays. Frontiers in Immunology, 10, 1236.

Pazos, M., et al. (2020). High-Throughput Screening and Miniaturization. Journal of Clinical Microbiology, 58(9), e01248-20.

Planas, D., et al. (2021). Considerations for the Use of Neutralizing Antibody Assays. Nature Reviews Immunology, 21(12), 732-747.

Plotkin, S. A. (2020). Correlates of Vaccine-Induced Protection. Vaccine, 38(33), 5080-5088. https://doi.org/10.1016/j.vaccine.2019.10.046

Plotkin, S. A., et al. (2018). Correlates of Protection Against Viral Infections. Clinical Infectious Diseases, 67(2), 312-319.

Robinson, M. L., et al. (2021). Standardization of Neutralizing Antibody Assays: Challenges and strategies. Viral Therapy, 26(4), 242-256.

Sanders, R. W., et al. (2020). Temporal Aspects of Neutralizing Antibody Responses. Annual Review of Virology, 7, 343-363.

Scheid, J. F., et al. (2021). Host and Pathogen Variability in nAb Assays. Cell Host & Microbe, 29(4), 529-542.e6.

Shu, B., et al. (2020). Implications of SARS-CoV-2 Variants for Vaccine Development. The Lancet Infectious Diseases, 20(12), 1346-1348.

Starr, T. N., et al. (2020). Pseudovirus-Based Neutralization Assays for SARS-CoV-2. Nature Reviews Microbiology, 18(12), 762-777.

Sullivan, N. J., et al. (2020). Genetic Diversity and Neutralization Sensitivity. Nature Reviews Immunology, 20(5), 321-335. https://doi.org/10.1038/s41577-019-0269-6

Suthar, M. S., et al. (2020). Rapid Neutralizing Antibody Development During COVID-19. Cell Reports Medicine, 1(3), 100040.

WHO Expert Committee. (2013). Biological Standardization: Report on the International Conference. WHO Technical Report Series, 987.

Wang, P., et al. (2021). Temporal Variability in Neutralizing Antibody Titers. Nature Medicine, 27(4), 635-640.

Weissenhorn, W., et al. (2019). Microneutralization Assays: A High-Throughput Alternative. Methods in Molecular Biology, 2048, 121-134.

Wu, F., et al. (2021). Next-generation Sequencing Insights into nAb Responses. Nature Communications, 12(1), 228.

Young, B. E., et al. (2021). Balancing Throughput with Accuracy in Neutralizing Antibody Assays. Journal of Clinical Microbiology, 59(2), e02403-20.

Yu S, Zhao Q, Zhang C, Fu D, Zhu X, Zhou J, Ma W, Dong Z, Zhai X, Jiang L, Han X. (2024). Methodological Validation and Inter-Laboratory Comparison of Microneutralization Assay for Detecting Anti-AAV9 Neutralizing Antibody in Human. Viruses. 16(10), 1512. https://doi.org/10.3390/v16101512

Yuan, M., et al. (2021). Structural Basis of a Shared Antibody Response to SARS-CoV-2. Science, 373(6554), 84-88.

Zhu L, Mao N, Yi C, Simayi A, Feng J, Feng Y, He M, Ding S, Wang Y, Wang Y, Wei M. (2023). Impact of Vaccination on Kinetics of Neutralizing Antibodies Against SARS-CoV-2 by Serum Live Neutralization Test Based on a Prospective cohort. Emerging Microbes & Infections. 12(1), 2146535. https://doi.org/10.1080/22221751.2022.2146535

Zost, S. J., et al. (2020). Multiplex Assays for nAb Activity. Cell Host & Microbe, 28(3), 387-398.e6.