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Development of an In-House SARS-CoV-2 Pseudovirus-Based Neutralization Assay: A Proof of Concept Study

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ABSTRACT The pseudovirus-based neutralization test (pVNT) is a valuable tool for detecting neutralizing antibodies against specific viruses, offering a safer and more versatile alternative to live virus assays. This project aimed to develop an in-house pVNT to detect neutralizing antibodies against SARS-CoV-2. A pseudovirus was engineered using a lentiviral system designed to express the wild-type SARS-CoV-2 spike protein and a GFP reporter gene. Key parameters for the assay, including cell number, pseudovirus dose, and detection time, were optimized. Performing this pVNT assay on SARS-CoV-2 convalescent and COVID-19 vaccinated sera comparison to plaque reduction neutralization test (PRNT) revealed a notably high correlation coefficient of 0.91. Moreover, the WHO International Standard for anti-SARS-CoV-2 immunoglobulin was tested in the pVNT to facilitate comparison of results in international units per milliliter (IU/mL). Our in-house pVNT demonstrates significant potential for detecting SARS-CoV-2 neutralizing antibodies and offers a promising alternative method for COVID-19 research. However, further studies are required to modify the pseudovirus spike protein to address ongoing variants of concern.

Keywords: SARS-CoV-2, COVID-19, Neutralizing antibodies, Pseudovirus, pVNT

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Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a profound impact on global health and economies.[1,2] As efforts to control the spread of the virus continue, it becomes crucial to understand the immune response to SARS-CoV-2 infection in order to develop effective vaccines, therapeutics, and public health strategies.[1] Among the various components of the immune response, neutralizing antibodies play a pivotal role in defending against viral infections.[3,4] These antibodies possess the ability to bind to specific viral antigens, such as the spike protein of SARS-CoV-2, thereby preventing viral entry into host cells and subsequent replication.[5] Measuring the presence and potency of neutralizing antibodies is essential for evaluating individual immune responses.[6,7]

Traditional live virus neutralization assays, such as plaque reduction neutralization assay, are considered the gold standard for measuring neutralizing antibodies against SARS-CoV-2.[8-11] However, they pose safety risks and require specialized facilities, limiting accessibility.[12]

To address these limitations, the pseudovirus-based neutralization test (pVNT) emerges as a promising alternative for evaluating neutralizing antibody responses to SARS-CoV-2.[13]

Pseudoviruses are engineered viral particles that express the protein of interest, in this case, the spike protein of SARS-CoV-2, on their surface, mimicking the entry process of the authentic virus.[14,15] These pseudoviruses are created using replication-deficient vectors, offering a safer and high-throughput platform for studying neutralizing antibody responses.[14,16] Various packaging systems have been employed to develop SARS-CoV-2 pseudoviruses, including vesicular stomatitis virus (VSV),[14,17] human immunodeficiency virus (HIV-1) lentivirus,[18,19] and murine leukemia viral (MLV) retrovirus systems.[20,21] Lentivirus systems are particularly favored due to their ability to efficiently incorporate large transgenes, like the full-length spike protein, and their high transduction efficiency in a broad range of cell types.[19,22]

The SARS-CoV-2 spike protein, specifically the S1 subunit containing the receptor-binding domain (RBD), is critical for viral attachment to the host cell receptor, ACE2, and is a key target for neutralizing antibodies.[6] As the virus evolves into different strains, mutations can occur in the RBD, altering its structure.[23] These changes can impact the virus's ability to infect host cells and its recognition by neutralizing antibodies. Therefore, it is essential to periodically update the spike protein used in pseudovirus neutralization test to reflect the viral strains of interest, ensuring an accurate assessment of neutralizing antibody responses.

While commercial services for SARS-CoV-2 pVNT exist, they often incur significant costs and require international importation. In this study, we aim to develop and preliminarily validate an in-house SARS-CoV-2 pVNT as a proof of concept. This work lays the foundation for establishing a reliable and cost-effective assay that can be locally implemented, potentially reducing future reliance on expensive, imported tests for assessing immune responses to SARS-CoV-2.

Materials and Methods

Cell culture and sample collection

For pVNT assay, HEK293T cells (American Type Culture Collection, USA) were cultivated in high-glucose DMEM (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), penicillin (100 IU/mL), and streptomycin (100 μg/mL) in a 5% CO_2 environment at 37°C. ACE2-overexpressing HEK293 cells (referred to as "ACE2-HEK293", American Type Culture Collection, USA) were maintained in MEM (Sigma-Aldrich, USA) supplemented with 10% FBS under similar conditions. Stable ACE2 expression was achieved through puromycin (Invivo, France) resistance selection. For PRNT assay, Vero cells (American Type Culture Collection, USA), were grown in the MEM solution supplemented with 10% FBS and 1% L-glutamine (Sigma, USA) under similar conditions.

The WHO International Standard anti-SARS-CoV-2 immunoglobulin (human standard antibody, NIBSC code: 20/136) was obtained from the National Institute for Biological Standards and Control (NIBSC). The concentration of this standard was 1,000 binding antibody units (BAU)/mL.[24] Serum samples (n = 52) were collected from SARS-CoV-2 convalescent patients (n = 34) and vaccinated individuals (n = 18) at the Department of Medical Science network hospital. Written informed consent was obtained under the approval of the Institution of the Development of Human Research Protection (IHRP) (COA No. IHRP2020056).

Pseudovirus production

Pseudoviruses carrying the spike protein of the SARS-CoV-2 wild-type strain were generated using the lentivirus packaging system (pPACK-SPIKE SARS-CoV-2, System Biosciences, USA).[25] Pseudovirus production was done following the manufacturer's protocol with minor optimization.[18] Briefly, the lentivirus structural plasmid, spike gene plasmid, and reporter plasmid 1. encoding green fluorescent protein (GFP) were co-transfected into HEK293 cells using Epipro (Polyplus transfection, France) as the transfection reagent. At 24 hours post-infection, SARS-CoV-2 pseudoviruses in the culture supernatants were harvested, filtered through a 0.45 μm pore size filter (Millipore, SLHP033RB), concentrated by ultracentrifugation at 5,000 g for 1 hour, and stored at -80°C until use.

The pseudovirus titer was determined by infecting ACE2-HEK293 cells with serial dilutions of the pseudovirus stock in a 96-well plate. After 3 days of infection, GFP-positive cells in each well were counted using fluorescence imaging (ImmunoSpot analyzers, USA) and analyzed with ImmunoSpot software (version 7.0.30.4). The viral titer was calculated based on the number of GFP-positive cells per milliliter.[16,26]

Pseudovirus neutralization test (pVNT)

Confirmation of the specificity of SARS-CoV-2 spike protein to ACE2 receptor

ACE2-HEK293 and HEK293T cells were seeded at a density of 5×10^3 cells per well in 96-well plates 24 hours prior to treatment.

Pseudovirus was added at a dose to produce approximately 750 GFP-positive cells per well (corresponding to a multiplicity of infection (MOI) of 0.15). After a 3-day incubation period, infection was assessed by observing GFP-positive cells under a fluorescence microscope (Nikon Eclipse Ti, USA).

Optimization of assay parameters

To determine the optimal incubation period for detecting SARS-CoV-2 pseudovirus infection, a time-course monitoring of GFP expression was conducted. Serial dilutions of the pseudovirus were administered to ACE2-293 HEK cells (1×10^4 cells per well), and infection efficiency was quantified at various time points (day 1 to day 5) by counting GFP-positive cells, using fluorescence imaging capture.

To optimize the ACE2-HEK293 cell number, a range of 5×10^3 to 3×10^4 cells per well was plated 24 hours prior to infection. SARS-CoV-2 convalescent sera were serially diluted in a two-fold stepwise manner from 1:20 to 1:5120 and incubated with pseudovirus at dose to produce approximately 750 GFP-positive cells per well at 37°C for 1 hour. The serum-virus mixture was then added to the ACE2-HEK293 cells. After 3 days of incubation, fluorescence imaging capture was employed to count the number of GFP-positive cells in each well. Neutralizing potency or serum inhibition rates were calculated based on the decrease in GFP-positive cells compared to non-serum controls. The 50% pseudovirus neutralization titer (pVNT50), defined as the serum dilution causing a 50% reduction in the number of GFP-positive cells, was determined using nonlinear regression analysis (log [inhibitor] vs. response [four parameters]) with GraphPad Prism 7.00 (GraphPad Software, Inc., San Diego, CA, USA).[27]

To optimize the pseudovirus dose, pVNT assays were conducted using pseudovirus doses to produce between 125 and 1,750 GFP-positive cells per well (corresponding to MOIs ranging from 0.0125 to 0.175). The pVNT assay was performed as described above, using ACE2-HEK293 cells at a fixed density of 1×10^4 cells/well. GFP-positive cells were counted, and pVNT50 values were calculated.

Plaque reduction neutralization test (PRNT)

The PRNT was developed by the Institute of Biological Products, Department of Medical Science [28] All steps involving live SARS-CoV-2 virus, including virus propagation and infection, were conducted in a Biosafety Level 3 (BSL3) facility for biosafety compliance. Vero cells (2×10^4 cells/well) were seeded and incubated at 37°C with 5% CO_2 for 24 hours. Serum samples were diluted (1:10, 1:40, 1:160, 1:640), and SARS-CoV-2 virus was prepared to produce 40 to 120 plaques per well. Equal volumes of diluted serum and virus were mixed and incubated at 37°C for 1 hour, then added to Vero cell monolayers. After 1 hour, the virus-serum mixture was replaced with a semisolid overlay medium (1% carboxymethylcellulose, 1% penicillin-streptomycin (Sigma-Aldrich, USA), and 10% fetal bovine serum), and plates were incubated for 7 days. After incubation, cells were fixed with 10% formaldehyde and stained with 0.5% crystal violet in PBS. Plaques were counted, and PRNT50 values were calculated

as the reciprocal of the highest serum dilution that resulted in a 50% plaque reduction compared to virus controls, using a four-point linear regression.

Preliminary validation of the pVNT

Precision

To determine the precision (reproducibility) of pVNT, serum from a single convalescent serum sample was tested 10 times across four independent experiments with 2 to 4 replications per plate. The intra-assay and inter-assay variability were evaluated, with acceptable coefficients of variation (%CV) set at ≤ 10%.[29]

Correlation between pVNT and PRNT

To assess the correlation between our pVNT and the standard PRNT, we compared neutralizing antibody titers measured by both assays using 52 serum samples and a NIBSC code 20/136. The correlation between neutralizing antibody titers from two methods was assessed using Pearson's correlation coefficient. Statistical analysis was performed using GraphPad Prism 7.0.

Initial comparison to WHO International Units

To make an initial comparison of pVNT titers to WHO International Units (IU/mL), the WHO International Standard (NIBSC code: 20/136) was tested during the validation process. The neutralization titers were compared, and a conversion factor was calculated to express titers in BAU/mL. This standardization allows for international comparability of the results.

Results

Specific interaction between the SARS-CoV-2 spike protein and ACE2 receptors

The number of GFP-positive cells in a well reflects the infection of cells by the pseudovirus. Transfection of the SARS-CoV-2 pseudovirus was confirmed by the presence of GFP-positive cells exclusively in ACE2-expressing HEK293 cells (Figure 1A, top panel), while HEK293T cells lacking ACE2 did not exhibit GFP fluorescence (Figure 1A, bottom panel). This indicates the absence of pseudovirus entry into cells without the ACE2 receptor, highlighting the specific interaction between the SARS-CoV-2 spike protein and ACE2 receptors. Consequently, ACE2-HEK293 cells were selected for neutralization assays, ensuring reliable results and specific assessment of the pseudovirus's interaction with the ACE2 receptor.

Optimal incubation period for SARS-CoV-2 pseudovirus detection

A time-course analysis of GFP expression from day 1 to day 5 post-infection with serial dilutions of the pseudovirus demonstrated a dose- and time-dependent increase in GFP-positive cells from day 1 to day 3. There was no significant increase in GFP-positive cells observed on days 4 and 5, suggesting a plateau in GFP expression. Therefore, day 3 post-infection was selected as the optimal time point for conducting the neutralization assay (Figure 1B).

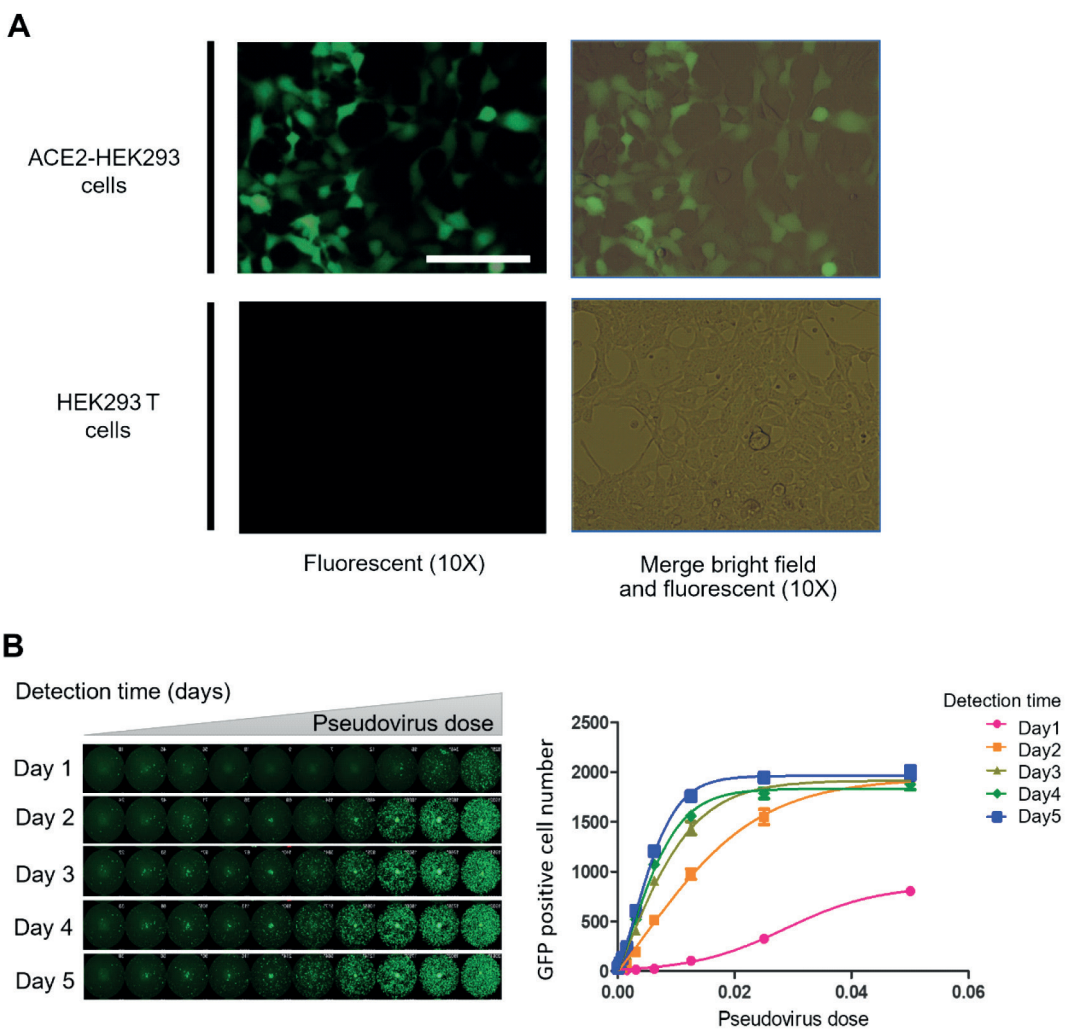
Optimal cell density for pVNT

Cell density optimization for the pVNT assay was performed using cell numbers ranging

from 5×10^3 to 3×10^4 per well. Goodness-of-fit analysis using four-parameter inhibition curves showed an R^2 value above 0.98 for cell densities up to 1×10^4 cells per well, indicating a robust fit (Figure 1C). Beyond this density, a decreasing trend in pRNT50 values was observed, with PRNT50 values dropping from 524.6 at 1×10^4 cells per well to 354.1, 268.1, and 255.4 for cell densities of 1.5×10^4, 2×10^4, and 3×10^4 cells per well, respectively. These results suggest that cell densities between 5×10^3 to 1×10^4 cells per well are suitable for the assay. For subsequent experiments, a cell density of 10,000 cells per well was selected to ensure consistency to prior experiment.

Optimal pseudovirus dose of SARS-CoV-2 pseudovirus in pVNT

Evaluation of different pseudovirus dose MOI ranging from 0.0125 to 0.175 was conducted to optimize the pseudovirus inoculation dose for the pVNT assay. Four-parameter inhibition curves with R^2 values above 0.98 were observed within the MOI range of 0.025 to 0.125. Consistent pVNT50 values were obtained for inoculant doses between MOI of 0.05 and 0.125, indicating stable neutralization results across this range (Figure 1D). Therefore, an intermediate inoculant dose with an MOI of 0.075 was selected. This MOI was chosen to ensure reliability while minimizing the risk of experimental errors or variability, and it will be used for future experiments.



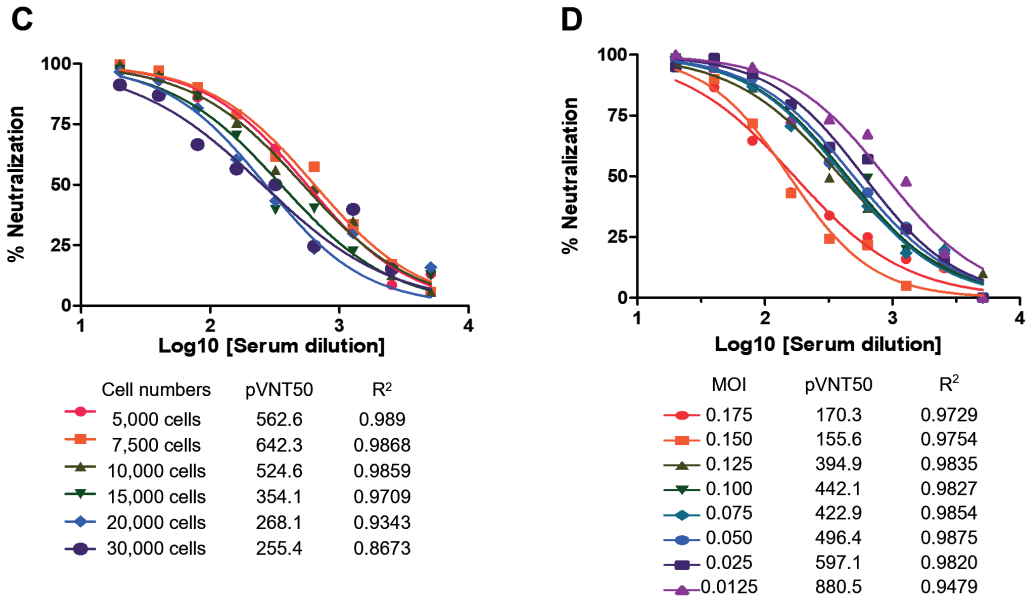


Figure 1 Establishment and optimization of the SARS-CoV-2 pVNT neutralization assay:

(A) Fluorescence microscope images of ACE2-HEK293 (top panel) and HEK293T cells (bottom panel) following infection with SARS-CoV-2 pseudovirus. GFP-positive cells, indicating successful pseudovirus transfection, were expressed only in ACE2-HEK293 cells, confirming the specificity of the pseudovirus entry through the ACE2 receptor. In contrast, no fluorescence was observed in ACE2-negative HEK293T cells. Representative images were captured at 10× magnification. Scale bar: 100 μm.

(B) Time-course analysis of GFP-positive cells in a 96-well plate following serial dilutions of the pseudovirus transfected into ACE2-HEK293 cells. GFP expression was quantified using fluorescence imaging capture to determine the optimal time point for detection post-transfection.

(C) Effect of varying ACE2-HEK293 cell densities on neutralization efficacy. The graph and accompanying table display cell numbers, pVNT50 values, and R^2 values for each cell density.

(D) Impact of different pseudovirus dose on neutralization efficacy. The graph and table present the pseudovirus dose, pVNT50 values, and R^2 values for each MOI.

Preliminary validation of the pVNT

We conducted preliminary validation of our in-house SARS-CoV-2 pVNT assay focusing on precision (reproducibility) and correlation to standard methods (PRNT). Additionally, we calibrated the assay results to WHO International Standard units for consistency.

Reproducibility was assessed by calculating intra-assay and inter-assay coefficients of variation (CVs), which were 7.64% and 8.17%, respectively (Table 1). These values fall within acceptable limits for cell-based assays, confirming the method's precision and reliability.[29]

Table 1 pVNT50 values of one COVID-19 convalescent patient measured across four independent experiments

	Replication						
Experiment no.	**1**	**2**	**3**	**4**	**Average**	**SD**	**CV**
Experiment 1	455.97	432.37	NA	NA	444.17	16.69	3.76
Experiment 2	366.93	331.33	NA	NA	349.13	25.17	7.21
Experiment 3	327.84	370.03	NA	NA	348.93	29.83	8.55
Experiment 4	672.18	867.13	718.93	754.14	753.10	83.11	11.04

NA: Not applicable or replications were not conducted

intra-assay CV = 7.64%

inter-assay CV = 8.17%

To examine the correlation of our pseudovirus neutralization assay with the standard method, we compared neutralizing antibody titers measured by our assay with those obtained using the PRNT in the same 53 serum samples including NIBSC code 20/136 (Table 2). The results showed a high correlation between the two assays, with a correlation coefficient of 0.9124, $R^2 = 0.83$, $p < 0.0001$ (Figure 2), indicating strong agreement between our in-house pVNT and the gold standard assays, PRNT.

Table 2 Neutralizing antibody titers of 52 serum samples from COVID-19 convalescent patients and vaccinated individuals and NIBSC 20/136 measured by pVNT and PRNT

	Sample	pVNT	PRNT		Sample	pVNT	PRNT
1	C01	565.33	645.57	24	C24	399.93	572.99
2	C02	252.09	161.66	25	C25	474.97	937.01
3	C03	3,189.20	2,874.74	26	C26	329.27	593.02
4	C04	1,597.76	1,280.00	27	C27	205.94	106.29
5	C05	1,803.80	1,280.00	28	C28	404.10	199.03
6	C06	1,280.54	1,280.00	29	C29	591.56	909.07
7	C07	436.60	383.35	30	C30	1,317.28	681.71
8	C08	833.93	1,280.00	31	C31	1,333.70	1,866.80
9	C09	1,535.72	1,280.00	32	C32	821.55	473.00
10	C10	2,035.41	1,280.00	33	C33	378.60	278.79
11	C11	152.78	235.88	34	C34	484.34	177.83
12	C12	237.97	205.35	35	VC01	808.44	693.81
13	C13	928.34	1,280.00	36	VC02	636.33	506.09
14	C14	529.43	287.47	37	VC03	318.28	422.32
15	C15	1,100.35	822.46	38	VC04	626.52	726.76
16	C16	1,986.05	1,600.00	39	VC05	866.55	576.29
17	C17	417.11	442.69	40	VC06	783.14	602.69
18	C18	1,180.10	1,212.27	41	VC07	234.48	182.10
19	C19	521.09	485.03	42	VC08	245.54	256.34
20	C20	2,478.83	2,874.74	43	VC09	1,113.34	956.15
21	C21	616.13	682.42	44	VC10	234.43	204.47
22	C22	313.04	331.97	45	VC11	283.09	327.58
23	C23	635.04	631.19				

	Sample	pVNT	PRNT
46	VC12	720.20	709.13
47	VC13	454.60	408.61
48	VC14	476.01	364.51
49	VC15	1,096.01	981.64
50	VC16	341.91	235.89
51	VC17	106.39	115.64
52	VC18	855.65	788.92
WHO International Standard			
1	NIBSC 20/136	2,090.52	1,450.00

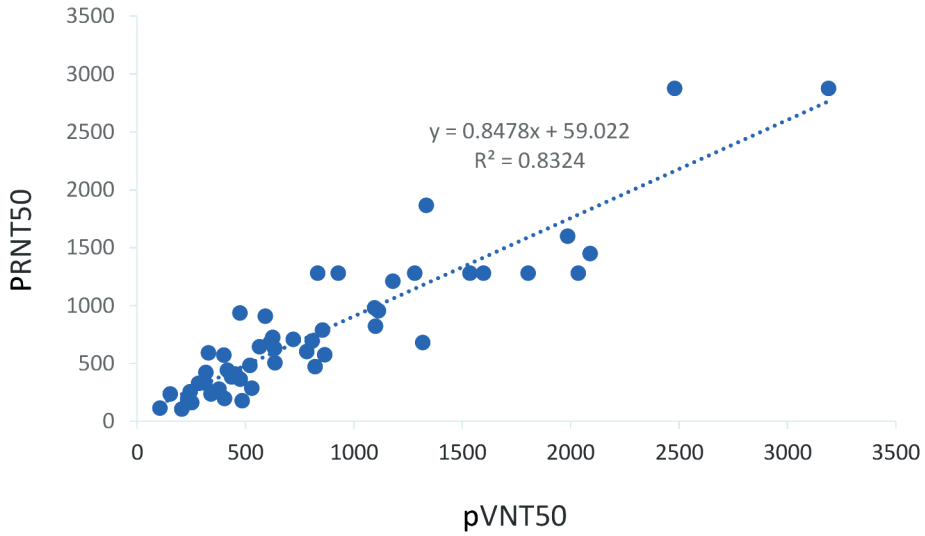


Figure 2 Correlation of neutralizing antibody titers from the pVNT and PRNT using 52 serum samples and NIBSC code 20/136. A strong positive correlation confirms the assay's reliability.

To ensure comparability of our assay results with international standards and other research groups, we determined the conversion factors of our pVNT using the WHO International Standard (NIBSC code 20/136), whose assigned concentration is 1,000 IU/mL. Our pVNT measurement for this standard was 2,090.52, resulting in a conversion factor of 0.4783 (1,000/2,090.52), which will be fully used for assay calibration and harmonization (PNT titer × 0.4783 = IU/mL).

Discussion

In this study, we successfully developed and validated an in-house pVNT for SARS-CoV-2, demonstrating its reliability and suitability as an alternative to traditional methods. The high correlation between neutralizing titers measured by our pVNT and the gold standard PRNT underscores the validity of our approach.

The pVNT employs pseudoviruses, which offer safety and flexibility benefits over live virus assays.[19,30-32] It can be managed in a Biosafety Level 2 (BSL-2) environment, reducing safety concerns and increasing accessibility. The pseudovirus system can be tailored to specific research objectives.[32] In our SARS-CoV-2 neutralization study, the spike protein on the surface of SARS-CoV-2, responsible for viral tropism through host cell recognition by binding to the human angiotensin-converting enzyme 2 (hACE2) receptor,[17,33,34] was used for

pseudotyping. This strategy effectively mimics the entry mechanism of the wild-type/live SARS-CoV-2 virus.[19,31] Although our current study focused on the wild-type strain of SARS-CoV-2, the assay can be readily adapted to evaluate neutralizing antibodies against emerging variants of the virus. This flexibility is crucial for keeping pace with the evolving virus and ensuring the continued relevance of the assay.

Reporter genes, such as luciferase,[31,35] and fluorescent proteins,[16,17] can be integrated into pseudoviruses for quantitative analyses. Luciferase assays offer lower background noise and higher sensitivity but are time-consuming and expensive. Fluorescent protein assays are cheaper and easier to operate.[13] The titers and transduction efficiency of pseudotyped viruses can be determined by counting fluorescence-positive cells using equipment such as a fluorescence microscope, an automatic fluorescence cell counter (e.g. ImmunoSpot analyzers), or a flow cytometer.[17] The choice of detection system depends on what is best for each lab's practice.

Developing the pVNT assay required optimizing several key parameters, including cell numbers, pseudovirus dose, and detection time. Previous studies have successfully implemented SARS-CoV-2 pVNTs using different cell models.[16,19,36,37] In our case, we confirmed that the spike-pseudotyped virus interacts effectively with ACE2-HEK293 cells, replicating the wild-type entry process. After the optimization, we selected a MOI of 0.075 for our assay. This balance between cell number and pseudovirus dose was crucial for obtaining reliable and reproducible results.

Our preliminary validation showed Intra-assay and inter-assay variations within the acceptable range for cell-based assays (CV ≤ 10%).[29] When using the WHO International Standard (NIBSC code: 20/136), our pVNT titer was 2,090, while another group reported a pVNT titer of 4,680.9.[38] Such variations in pVNT titers across different laboratories are not unexpected, given the potential differences in experimental conditions and pseudovirus designs. Nevertheless, calibration against international standards enables these differences to be normalized, facilitating meaningful comparisons between studies.

Accurate measurement of neutralizing antibodies is vital for developing effective vaccines and therapeutic interventions. While our "Proof of Concept" study lays a strong foundation, our preliminary validation used a relatively small sample size. This was primarily due to restricted access to clinical samples, which arose from challenges such as the availability of suitable cohorts, regulatory approvals, and high demand during the pandemic. Further validation with larger cohorts will enhance the assay's reliability and robustness. Additional research is needed to expand the assay's applicability. Collaborative efforts could help standardize the pVNT assay, promoting broader adoption and ensuring consistency across different laboratories.

Conclusion

An in-house pVNT for SARS-CoV-2 was successfully developed and optimized, with key parameters such as cell number, pseudovirus dose, and incubation time refined. The assay

demonstrated good precision and a high correlation with the gold standard live virus assay, underscoring its reliability. These results suggest that the pVNT could serve as a promising alternative to traditional neutralization methods, with the potential for future use in monitoring vaccine efficacy and evaluating treatments.

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การพัฒนาการตรวจหาแอนติบอดีลบล้างฤทธิ์ไวรัส SARS-CoV-2 ในห้องปฏิบัติการด้วยวิธี Pseudovirus Neutralization Test: การศึกษาเชิงพิสูจน์แนวคิด

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บทคัดย่อ Pseudovirus-based neutralization test (pVNT) เป็นวิธีในการตรวจหาแอนติบอดีที่ลบล้างฤทธิ์ไวรัส โดยใช้ pseudovirus แทนการใช้ไวรัสจริง ซึ่งเป็นวิธีที่มีความปลอดภัยสูงและสามารถปรับใช้งานได้หลากหลาย งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาวิธี pVNT สำหรับตรวจหาระดับแอนติบอดีที่ลบล้างฤทธิ์ไวรัส SARS-CoV-2 โดยใช้ pseudovirus ที่สร้างจาก lentivirus ซึ่งถูกออกแบบให้แสดงโปรตีนหนามของ SARS-CoV-2 สายพันธุ์ wildtype และโปรตีนเรืองแสงสำหรับรายงานผล ในการทดลองทำการทดสอบปัจจัยต่าง ๆ ที่สำคัญ เช่น ปริมาณเซลล์เป้าหมาย ปริมาณ pseudovirus และระยะเวลาในการตรวจสอบผล เมื่อนำไปทดสอบกับตัวอย่างซีรัมของผู้ที่เคยได้รับเชื้อโควิดและผู้ที่ได้รับวัคซีน เปรียบเทียบผลกับวิธี plaque reduction neutralization test (PRNT) พบว่ามีค่าความสัมพันธ์สูง โดยมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.91 จากการตรวจสอบความแม่นของการทำซ้ำ พบค่าสัมประสิทธิ์ความแปรปรวนในชุดวิเคราะห์เดียวกันและความแปรปรวนต่างชุดวิเคราะห์ คือ 7.64% และ 8.17% ตามลำดับ นอกจากนี้ทำการทดสอบวิธี pVNT โดยใช้ตัวอย่างแอนติบอดีต้าน SARS-CoV-2 มาตรฐานสากลขององค์การอนามัยโลกเพื่อเปรียบเทียบค่าเป็นหน่วยสากล (WHO International Units; IU/mL) ดังนั้นวิธี pVNT ที่พัฒนาขึ้นนี้จึงเป็นวิธีที่สามารถนำไปใช้ตรวจหาแอนติบอดีที่ลบล้างฤทธิ์ไวรัส SARS-CoV-2 ได้ เป็นอีกทางเลือกสำหรับงานวิจัยที่เกี่ยวข้องกับโรคโควิด 19 อย่างไรก็ตามต้องมีการศึกษาเพิ่มเติมและปรับปรุงโปรตีน spike ของ pseudovirus เพื่อให้สอดคล้องกับสายพันธุ์ที่สนใจในปัจจุบัน

คำสำคัญ: ซาร์สโควีทู, โควิด 19, แอนติบอดีลบล้างฤทธิ์, ไวรัสเทียม, วิธี pVNT