Development of a novel hybrid alphavirus-SARS-CoV-2 particle for rapid in vitro screening and quantification of neutralization antibodies, antiviral drugs, and viral mutations

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SUMMARY

Timely development of vaccines and antiviral drugs are critical to control the coronavirus disease 2019 (COVID-19) global pandemic 1-6. Current methods for validation of vaccine efficacy involve the use of pseudoviruses, such as the SARS-CoV-2 spike protein (S) pseudotyped lentivirus or vesicular stomatitis virus (VSV), to quantify neutralizing antibodies for blocking viral infection 7-14. The process of pseudovirus infection and quantification is time consuming and can take days to complete. In addition, pseudoviruses contain structural proteins not native to SARS-CoV-2, which may alter particle properties in receptor binding and responses to antibody neutralization 15. Here we describe the development of a new hybrid alphavirus-SARS-CoV-2 particle (Ha-CoV-2) for rapid screening and quantification of neutralization antibodies and antiviral drugs. Ha-CoV-2 is a non-replicating SARS-CoV-2 virus-like particle, composed of only SARS-CoV-2 structural proteins (S, M, N, and E) and a RNA genome derived from a fast expressing alphavirus vector 16. We demonstrate that Ha-CoV-2 can rapidly and robustly express reporter genes in target cells within 3-5 hours following viral entry. We further validate the Ha-CoV-2 system for rapid quantification of neutralization antibodies and antiviral drugs. In addition, we assembled a Ha-CoV-2 particle bearing the D614G mutant spike protein, and found that the mutation led to an approximately 200% increase in virion infectivity. These results demonstrate that Ha-CoV-2 can also be applied for rapid monitoring and quantification of viral mutations for effects on neutralizing antibodies induced by vaccines.
INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a rapidly spreading, novel beta-coronavirus that is causing the ongoing global pandemic of coronavirus disease 2019 (COVID-19)\(^{17-21}\). SARS-CoV-2 has caused over 76 million infections and 1.7 million deaths globally as of December 2020. Antiviral drugs and neutralizing antibodies are expected to be effective to combat the pandemic. In particular, neutralizing antibodies, induced by vaccines or by the virus, can play a critical role in controlling and preventing infection. Currently, only one FDA-approved drug, remdesivir, is available to reduce hospital stay\(^1\); no effective drug is available to lower mortality. Several vaccine candidates have recently shown significant results in phase III clinical trials\(^2-6\), and have been approved or in the approval process for emergency use. Nevertheless, the effectiveness of vaccines needs to be continuously monitored for the induction of neutralizing antibodies against evolving viral mutants in circulation.

Current antiviral drug screening and quantification of neutralizing antibodies rely on the use of SARS-CoV-2 pseudoviruses\(^7-14\). The use of live virus requires biosafety level (BSL) 3 facility and practice, which limits the use of wild-type virus in common laboratories. Both lentivirus and vesicular stomatitis virus (VSV), pseudotyped with the SARS-CoV-2 S protein, are used in cell-based neutralization assays and in antiviral drug screening\(^8-11\). SARS-CoV-2 contains four structural proteins: the spike protein (S), the membrane protein (M), the envelope protein (E), and the nucleocapsid protein (N)\(^22,23\). S is the major viral protein responsible for virus attachment and entry to the target cells\(^24-26\), and thus, is commonly used to pseudotype viruses. The process of pseudotyping involves the use of a reporter genome and the capsid proteins of lentivirus or VSV. In practice, lenti-based pseudoviruses are produced by co-transfection of vectors encoding the lentiviral gag-pol structure proteins, a reporter genome, and the S protein\(^10\).
The VSV-based pseudovirus is generated by transfection of a producer cell line with a SARS-CoV-2 S expression vector, and then followed by infection of the cell with a VSV reporter virus that lacks the VSV major spike glycoprotein (G). The VSV-based pseudovirus can generate a higher signal-to-noise ratio and the neutralization assay takes about 24 hours, whereas the lentivirus-based pseudovirus produces less robust signal and takes 48-72 hours\(^7\text{–}^{11}\). However, a major issue for the VSV-based pseudovirus is the likely presence of residual VSV virus, which may result in high rates of false-positive results\(^{27}\). In addition, both VSV- and lentiviral-based pseudoviral particles contain structural components not native to the wild-type SARS-CoV-2, which may alter particle properties in receptor binding and responses to antibody neutralization\(^{15}\).

To overcome the shortcomings of pseudoviruses, here we describe the development and validation of a new hybrid alphavirus-CoV-2 particle (Ha-CoV-2) for rapid quantification of neutralization antibodies and antiviral drugs. Ha-CoV-2 is a non-replicating SARS-CoV-2 virus-like particle, composed of authentic virus structural proteins (S, M, N, and E) from SARS-CoV-2 but not of structural proteins from any other virus. Ha-CoV-2 also contains a genome derived from an alphavirus-based vector\(^{16,28}\); the alphavirus-based genome of Ha-CoV-2 can rapidly and robustly express reporter genes within hours after viral entry\(^{28}\). In this study, we further demonstrate that Ha-CoV-2 can be used as a platform for rapid quantification of neutralization antibodies, antiviral drugs, and viral structural protein mutations.

**RESULTS**

To establish a rapid cell-based SARS-CoV-2 infection system for screening and evaluation of neutralizing antibodies and antiviral drugs, we developed a new hybrid alphavirus-SARS-CoV-2
viral particle, in which an alphavirus-based RNA genome is enclosed for rapid expression of reporter genes in target cells (Fig. 1A). The genomic RNA consists of the 5’ untranslated region and open-reading frames coding for the nonstructural proteins (nsp) 1-4 from Semliki Forest virus (SFV); the inclusion of nsp1-4 allows for self-amplification of the RNA genome in cells. The RNA genome also contains viral subgenomic RNA promoters for the expression of reporter genes (such as luciferase). The 3’ end of the genome contains the 3’ untranslated region of SFV and a poly(A) tail that are used to stabilize RNA. In addition, a putative SARS-CoV-2 packaging signal was inserted downstream of the reporter gene to facilitate RNA packaging by the SARS-CoV-2 structural protein N.

To assemble viral particles, we used a DNA vector, Ha-CoV-2, to express the genomic RNA. Ha-CoV-2 was cotransfected with vectors expressing the structural proteins of SARS-CoV-2 (S, M, E, and N) into HEK293T cells (Fig. 1A). Virion particles were harvested at 48 hours post cotransfection, and tested for virion infectivity and the ability to express reporter genes in target cells. First, to confirm the presence of the SARS-CoV-2 structural proteins in Ha-CoV-2 particles, we performed western blots of purified particles, using antibodies against the S protein of SARS-CoV-2. We were able to detect the presence of S in Ha-CoV-2 particles (Fig. 1B). To further determine the presence of the other structural proteins of SARS-CoV-2, we also assembled Ha-CoV-2 particles using FLAG-tagged M and N proteins, and performed western blots using anti-FLAG antibodies. We were able to confirm the presence of both M and N in Ha-CoV-2 particles (Fig. 1C and 1D). Furthermore, to determine whether these structural proteins are present in the same virion particles, we used anti-S antibody-conjugated magnetic beads to pull down Ha-CoV-2 particles. The magnetically separated particles were further probed with western blot using the anti-FLAG antibody for the presence of FLAG-tagged M protein. As
shown in Fig. 1E, we detected the presence of M in the anti-S antibody pull-down particles, confirming that cotransfection of the SARS-CoV-2 structural proteins with Ha-CoV-2 vector led to the production of SARS-CoV-2 virus-like particles (VLPs).

To further demonstrate the ability of Ha-CoV-2 particles to infect and express reporter genes in target cells, we assembled an Ha-CoV-2(GFP) reporter virus, and used it to infect HEK293T(ACE/TEMPRSS2) cells that overexpressed both ACE2 and TMPRSS2. We observed GFP expression in cells following infection (Fig. 2A), demonstrating that the alphavirus-based RNA genome can be packaged by the budding SARS-CoV-2 VLPs, and is capable of expressing the GFP reporter gene in target cells. To determine whether the infection of target cells by Ha-CoV-2 is dependent on the interaction of S with the ACE2 receptor 18,30, we assembled an Ha-CoV-2(Luc) reporter virus and used it to simultaneously infect HEK293T(ACE/TEMPRSS2) cells and the parent HEK293T cell that does not express ACE2. As shown in Fig. 2B, Ha-CoV-2(Luc) expressed Luc only in the HEK293T(ACE/TEMPRSS2) cells but not in HEK293T, demonstrating the requirement of ACE2 for Ha-CoV-2 infection. We further confirmed the requirement for S-ACE2 interaction for Ha-CoV-2 infection by generating particles without the S protein. As shown in Fig. 2C, in the absence of S, Luc expression was highly diminished, demonstrating the requirement of S for Ha-CoV-2 infection. We also tested the requirement for the other structural proteins, M, N, and E, for Ha-CoV-2 infection. Although these proteins were found to be nonessential, removal of M, N, and E led to a reduction in Ha-CoV-2 infectivity (Fig. 2C). We further investigated the individual contributions of M, N, and E for Ha-CoV-2 infection. It appeared that in general, particles generated with two or three of these structural proteins had a greater infectivity than those with only one protein. However, the presence of S plus E appears to be sufficient for the full infectivity of Ha-CoV-2 (Fig. 2D).
Lenti-based SARS-CoV-2 pseudoviruses have been commonly used for antiviral drug screening and neutralization antibody assays. We performed a comparison of Ha-CoV-2 with lenti-pseudovirus for the infection of both ACE2 overexpression cells and cells expressing native levels of ACE2. Lenti-pseudovirus and Ha-CoV-2 particles were assembled in similar cell culture conditions, and an equal volume of the particles produced was used for infection. Both lenti-pseudovirus and Ha-CoV-2 can infect the ACE2-overexpressing HEK293T(ACE2/TMPRESS2) cells. However, infection of Calu-3, a human lung cancer cells expressing native levels of ACE2, was minimal with the lenti-pseudovirus, whereas Ha-CoV-2 particles produced a more robust signal for the infection of Calu-3 cells. Infection of primary human ACE2-null monkey kidney cells with Ha-CoV-2 did not generate signals above uninfected cell background. These results demonstrate that Ha-CoV-2 is likely more sensitive for infecting low ACE2 expression cells.

A major advantage of utilizing alphavirus-based RNA genome for Ha-CoV-2 is the extremely fast speed and high-level gene expression of alphaviruses; gene expression from the subgenomic RNA promoters occur within hours of infection, and levels of viral plus-RNAs can reach 200,000 copies in a single cell. We followed the time course of Ha-CoV-2(Luc) infection, and observed that the Luc reporter expression increased rapidly within 6 hours from the addition of particles to cells. This rapid reporter expression kinetics permitted us to utilize Ha-CoV-2 for fast screening and quantification of neutralization antibodies and anti-viral drugs.

To validate the Ha-CoV-2 system for rapid screening and quantification of neutralizing antibodies, we tested an anti-SARS-CoV-2 antiserum (1F), which was serially diluted and pre-incubated with Ha-CoV-2(Luc). The antibody-virus complex was used to infect cells for 5 hours.
for Luc expression. As shown in Fig. 5A, we observed 1F concentration-dependent inhibition of Ha-CoV-2(Luc), and the IC50 was determined to be at 1:433 dilution (Fig. 5A). Given that SARS-CoV-2 lenti-pseudoviruses have been widely used in neutralization assays\(^4,8,14\), we also performed a similar assay using 1F and a lenti-pseudovirus, Lenti-SARS-CoV-2(Luc)\(^{15}\). Infected cells were analyzed at 72 hours post infection. We observed similar 1F concentration-dependent inhibition of the lenti-pseudovirus, and the IC50 was found to be at 1:186 dilution (Fig. 5B). These results demonstrated that Ha-CoV-2 is as effective as lenti-pseudoviruses for quantifying neutralizing antibodies, but with a much faster speed (5 hours versus 48-72 hours).

Based on the 1F antiserum results described above, we performed additional Ha-CoV-2 antibody neutralizing assays using anti-sera from COVID-19 patients. We observed antibody concentration-dependent inhibition of Ha-CoV-2 infection in all these serum samples. The inhibition curves and IC50s of each serum are presented in Fig. 5C. Among these samples, the 20M anti-serum has the most potent neutralizing activity with an IC50 at 1:1470 dilution. For comparison, an independent study was conducted using infectious SARS-CoV-2 to validate these anti-sera. We confirmed neutralizing activities from all of them, and the 20M anti-serum was also found to be the most potent one (Fig. 5D). In addition, there is a direct correlation in the IC50s obtained from the Ha-CoV-2 system and those obtained from infectious SARS-CoV-2 (Fig. 5D). These results demonstrated that Ha-CoV-2 can be used for rapid quantification of the neutralizing activities of anti-sera.

Pseudoviruses have been commonly used for high throughput screening of SARS-CoV-2 entry inhibitors\(^7,10\). We tested a broad-spectrum viral entry inhibitor, Arbidol (Umifenovir)\(^{32}\), for its ability to block Ha-CoV-2(Luc) infection. As shown in Fig. 5E, we observed dosage-dependent inhibition of Ha-CoV-2(Luc) in 5 hours, and the IC50 was determined to be 16 µM.
These results demonstrated that Ha-CoV-2 can be used for rapid screening of SARS-CoV-2 entry inhibitors.

Finally, we tested whether the Ha-CoV-2 system can be used for rapid evaluation of the relative infectivity of viral mutants. The D614G spike mutation emerged early in the COVID-19 pandemic, and has recently been reported to confer greater infectivity that has led to the global dominance of the D614G mutant in circulation\textsuperscript{33,34}. To determine whether the increase in virus infectivity can be recapitulated and quantified by the Ha-CoV-2 system, we assembled Ha-CoV-2 particles using the G614 mutant S protein (614G) or the parental D614 S protein. We found that D614G mutation did not increase virion release or the level of S protein virion incorporation (\textbf{Fig. 5G} and \textbf{5H}). However, Ha-VoV-2 particles bearing the G614 spike were found to be nearly 3 times more infectious than those bearing the D614 spike (\textbf{Fig. 5I}). These results demonstrated that the Ha-CoV-2 system can provide a convenient tool for rapid monitoring and quantification of viral mutations for potential impacts on neutralizing antibodies and vaccine effectiveness.

\textbf{DISCUSSION}

The study of SARS-CoV-2 requires high-level containment that limits the use of wild-type virus in common clinical and research laboratories. Pseudoviruses and virus-like particles (VLPs) have been widely used for SARS-CoV-2 drug discovery and vaccine development. Pseudoviruses, such as those derived from lentivirus and vesicular stomatitis virus, can mimic the entry process of SARS-CoV-2. However, structurally, they are not identical to SARA-CoV-2 and lack structural components provided by M, E, and N of SARS-CoV-2. VLPs closely resemble SARS-CoV-2 particles, but VLPs contain no genome for reporter expression in target cells\textsuperscript{35}. In this article, we described the development and validation of a novel hybrid system, the Ha-CoV-2
particle, which is structurally a VLP, but possesses the ability of a pseudovirus to enter and express reporter genes in target cells. The genome of Ha-CoV-2 is derived from alphavirus, which allows for rapid and robust quantification of reporter expression within hours of viral entry. We further demonstrated that Ha-CoV-2 can be used for rapid screening and quantification of neutralization antibodies and antiviral drugs.

We also performed a direct comparison between Ha-CoV-2 and a lenti-pseudovirus in antibody neutralization assays. While both systems are effective in quantifying neutralizing antibodies, the sensitivity of the two systems may differ. Pseudoviruses contain only the S protein of SARS-CoV-2, whereas Ha-CoV-2 contains all four structural proteins (S, M, E, and N) of SARS-CoV-2, and has no structural proteins from other viruses (e.g. gag and pol of lentivirus). Although S is the primary requirement for viral entry, the presence of other structural proteins of SARS-CoV-2 may also affect virion infectivity and particle interaction with cell membrane and antibodies. In our system, the lack of M and E on virion particles does appear to lower virion infectivity (Fig. 2D).

In addition to viral structural proteins, virion particles also incorporate multiple cellular proteins during virion budding and release. Many of these cellular factors such as PSGL-1 can impact virion infectivity\textsuperscript{15,36-38} and antibody binding to plasma membrane\textsuperscript{39}. SARS-CoV-2 budding occurs mainly at the membranes of ER-Golgi intermediate compartment\textsuperscript{40}, whereas pseudoviruses such as lenti-pseudoviruses bud from the plasma membrane\textsuperscript{41}. Because of the differences, it is possible that different sets of cellular proteins may be incorporated into pseudoviruses and SARS-CoV-2. In this regard, the close resemblance of Ha-CoV-2 particle to SARS-CoV-2 may provide a unique tool for studying effects of virion host proteins in SARS-CoV-2 infection and pathogenesis\textsuperscript{15}.
As SARA-CoV-2 continue to circulate and evolve, it may pose a particular challenge for the control of the COVID-19 pandemic, as documented in the recent emergence of the B.1.1.7 lineage in UK.\textsuperscript{42} Viral mutation may lead to increases in viral transmission and fitness, and thus there is an urgent need for rapid identification and characterization of emerging mutants for changes in infectivity and evasion of neutralizing antibodies. The Ha-CoV-2 system can be readily optimized for such purposes.
Materials and Methods

Virus and viral particle assembly

The SARS-CoV-2 S, S(D614G), M, E, or N expression vectors were purchased from Sinobiological. The Ha-CoV-2(Luc) and Ha-CoV-2(GFP) vectors were synthesized by Twist Bioscience. Ha-CoV-2 particles were assembled by cotransfection of HEK293T cells in 10 cm dish with 2.5 µg of each of the SARS-CoV-2 structural protein expression vectors (S, N, E, M) and 10 µg of Ha-CoV-2(Luc) or Ha-CoV-2(GFP). Particles were harvested at 48 hours post cotransfection, filtered through a 0.45 µm filter, and then concentrated by gradient centrifugation. Lenti-pseudovirus was assembled by cotransfection of HEK293T cells with SARS-CoV-2 S expression vector (0.5 µg), pCMVΔR8.2 (7.5 µg), and pLTR-Tat-IRES-Luc (10 µg) as previously described \(^\text{15}\).

Detection of Ha-CoV-2 virion incorporation of structural proteins

The SARS-CoV-2 M-FLAG and N-FLAG vectors were kindly provided by Dr. Pei-Hui Wang \(^\text{43}\). HEK293T cells were co-transfected with 10 µg Ha-CoV-2(Luc), 2.5 µg of the SARS-CoV-2 S expression vector, and 2.5 µg each of the M-FLAG, N-FLAG, and E-FLAG vectors. Particles were harvested, filtered through a 0.45 µm filter, and then purified by gradient centrifugation. Virion lysates were analyzed by SDS-PAGE and western blot using Spike Protein S2 Monoclonal Antibody (1A9) (Invitrogen) (1:1000 dilution) or DYKDDDDK Tag Monoclonal Antibody (FG4R) (Invitrogen) (1: 1000 dilution). Membranes were then incubated with Anti-mouse IgG, HRP-linked Antibody (Cell signaling) (1: 2000 dilution) for 60 min at room temperature. Chemiluminescence signal was detected by using West Pico or West Femto chemiluminescence reagent (Thermo Fisher Scientific). Images were captured with a CCD camera (FluorChem 9900 Imaging Systems) (Alpha Innotech). Particles were also captured with
magnetic beads for analyses. Briefly, magnetic Dynabeads Pan Mouse IgG (Invitrogen) (2x10^7 beads/50 μl) were conjugated with Spike Protein S2 Monoclonal Antibody (1A9) (Invitrogen) (2 μl antibody) for 30 minutes at room temperature. After conjugation, virions were incubated with the anti-S2-beads for 30 minutes at 4°C, and pulled down with a magnet. After washing with cold PBS for 5 times, virions were lysed in LDS lysis buffer (Invitrogen). Lysates were analyzed by SDS-PAGE and western blot using D Y K D D D D D K Tag Monoclonal Antibody (FG4R) (Invitrogen) (1: 1000 dilution) to detect FLAG-Tagged SARS-CoV-2 M proteins.

**Viral infectivity assay**

Ha-CoV-2 particles were used to infect HEK293T(ACE2/TMPRSS2) cells (a gift from Virongy LLC, Manassas, VA), Calu-3 cells (ATCC), HEK293T cells (ATCC) and primary monkey kidney cells provided by Dr. Xuefeng Liu. Briefly, cells were seeded in 12-well plates (2x10^5 cells) per well. Cells were infected for 1-2 hours at 37°C, washed, cultured in fresh medium for 3-48 hours, and then lysed in Luciferase Assay Lysis Buffer (Promega) for luciferase activity using GloMax Discover Microplate Reader (Promega). Lenti-pseudovirus particles were used to infect HEK293T(ACE2/TMPRSS2) cells and Calu-3 cells (ATCC). Cells were infected for 2 hours, cultured for 3 days, and then lysed in Luciferase Assay Lysis Buffer (Promega) for luciferase assays using GloMax Discover Microplate Reader (Promega).

**Neutralizing Antibody Assay**

Ha-CoV-2 particles were pre-incubated with serially diluted sera from COVID19 patients for 1 hour, and then added to HEK293T(ACE2/TMPRSS2) cells for 2 hours. Cells were then washed, and cultured in fresh medium for additional 3 hours. Cells were lysed in Luciferase Assay Lysis Buffer (Promega) for luciferase assays using GloMax Discover Microplate Reader (Promega). For neutralization assays using wild-type SARS-CoV-2 virus, anti-serum was serially diluted (a
twelve-point, two-fold dilution series starting at 1:10 dilution), and pre-incubated with 100 pfu of SARS-CoV-2 for 1 hour at 37 °C. After incubation, viral plaque assay was conducted to quantify viral titers. Briefly, Vero cells (ATCC) in 12-well plates (2x10⁵ cells per well) were infected with virus for 1 hour at 37 °C. After infection, a 1:1 overlay, consisting of 0.6% agarose and 2X EMEM without phenol red (Quality Biological), supplemented with 10% fetal bovine serum (FBS) (Gibco), was added to each well. Plates were incubated at 37°C for 48 hours. Cells were fixed with 10% formaldehyde for 1 hour at room temperature, and then the agarose overlay was removed. Cells were stained with crystal violet (1% CV w/v in a 20% ethanol solution). Viral titer of SARS-CoV-2 was determined by counting the number of plaques.

**Antiviral Drug Assay**

Arbidol-hydrochloride (Sigma) was resuspended in Dimethyl sulfoxide (Sigma). HEK293T(ACE2/TMPRSS2) cells were pretreated for 1 hour with serially diluted Arbidol. Ha-CoV-2 particles were added cells, followed by the addition of Abidol to maintain the drug concentration. Cells were infected in the presence of Arbidol for 2 hours, washed, and then cultured in fresh medium for a total of 5 hours. Cells were lysed in Luciferase Assay Lysis Buffer (Promega) for luciferase assays using GloMax Discover Microplate Reader (Promega).

**Data availability**

All data generated or analyzed during this study are included in this article.
REFERENCES


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AUTHOR CONTRIBUTIONS


DECLARATION OF INTERESTS

A patent application has been filed by George Mason University.
Fig. 1. Design and assembly of Ha-Cov-2 particles. (A) Illustration of the design of Ha-CoV-2 vector. The vector contains a RSV promoter that transcribes the full-length viral RNA genome to be packaged into Ha-CoV-2 particles. Shown are the 5’ untranslated region followed by open-reading frames coding for nonstructural proteins (nsp) 1-4 from Semliki Forest virus (SFV), viral subgenomic promoters for Luc and GFP reporter expression, the 3’ untranslated region and a poly(A) tail that is self-cleaved by the hepatitis delta virus ribozyme (RZ). The SARS-CoV-2 packaging signal is inserted in front of the 3’ untranslated region. To assemble viral particles, HEK293T cells were co-transfected with Ha-CoV-2 and the vectors expressing the 4 structural proteins of SARS-CoV-2 (S, M, E, and N). HA-CoV2 particles in the supernatant was harvested at 48 hours, purified, lysed, and then analyzed by western blot using antibodies for the SARS-CoV-2 S protein (B). Control is supernatants from cells transfected with the Ha-CoV-2 vector alone. (C and D) Particles were also assembled using FALG-tagged M and N. Particles were analyzed with western blot using an antibody against FLAG. (E) Particles in the supernatant were also captured with magnetic beads conjugated with the anti-S antibody, and then analyzed with western blot using the antibody again FLAG for FLAG-tagged M protein in the particles.
Fig. 2. SARS-CoV-2 S protein and ACE2-dependent infection of target cells by Ha-CoV-2. (A) HEK293T(ACE2/TMPRSS2) cells were infected with Ha-CoV-2(GFP) particles. GFP expression was observed 48 hours post infection. (B) ACE2-dependent infection of target cells by Ha-CoV-2(Luc). HEK293T(ACE2/TMPRSS2) and HEK293T cells were infected with Ha-CoV-2(Luc) particles. Luciferase expression was quantified at 24 hours post infection. (C) SARS-CoV-2 S protein-dependent infection of target cells by Ha-CoV-2(Luc). Particles were assembled in the presence or absence of S or M + E + N. Luciferase expression was quantified at 4 hours post infection. (D) Requirements of M, E, and N for the optimal infectivity of Ha-CoV(Luc). Particles were assembled in the presence S and combinations of individual proteins of M, E and N. Luciferase expression was quantified. Assays in (B) to (D) were performed in triplicates.
Fig. 3. Comparison of SARS-CoV-2 S pseudotyped lentivirus with Ha-CoV-2 particles. Calu-3 and HEK293T(ACE2+TMPRSS2) cells were infected with an equal volume of viral particles, Lenti-CoV-2(Luc) or Ha-CoV-2(Luc). Relative infection was quantified by luciferase assay at 72 hours post infection. Primary monkey kidney cells were also infected with Ha-CoV-2 for comparison. All assays were performed in triplicates.
Fig. 4. Rapid time course of reporter gene expression in Ha-CoV-2(Luc) infection.

A 6-hour time-course of luciferase reporter expression following infection of HEK293T(ACE2+TMPRSS2) cells with Ha-CoV-2(Luc) particles. Cells were infected with Ha-CoV-2(Luc) for 2 hours, washed, cultured in fresh medium, and then lysed and analyzed for Luc expression at different time points. The addition of virus to cells was defined as time “0”. Assays were performed in triplicates.
Fig. 5. Validation of Ha-CoV-2 particles for rapid screening and quantification of neutralizing antibodies and antiviral drugs. (A) Quantification of neutralizing antibodies with Ha-CoV-2 particles. Shown are the concentration-dependent inhibition of Ha-CoV-2(Luc) by the anti-serum 1F and the 1F inhibition curve. 1F was serially diluted and incubated with Ha-CoV-2(Luc) particles for 1 hour at 37°C. The Ha-CoV-2(Luc)-antibody complex was used to infect HEK293T(ACE2/TMPRSS2) cells. Neutralization activities were quantified by luciferase assay at 5 hours post addition of virus to cells. Control serum was from healthy, uninfected donors. The IC50 was calculated using the relative percentage of infection versus serum concentration. (B) For comparison, the anti-serum 1F was also similarly quantified using a SARS-CoV-2 S protein pseudotyped lentivirus, Lenti-CoV-2(Luc). Neutralization activities were quantified with
luciferase assay at 72 hours post infection. (C) Rapid quantification of anti-sera (1F, 8F, 19F and 20M) from COVID-19 patients using Ha-CoV-2(Luc) particles. The inhibition curves were generated similarly to (A). (D) Quantification of anti-sera using infectious SARS-CoV-2 virus. Neutralization activities were similarly quantified and the IC50s were calculated. Correlation in IC50s obtained from SARS-CoV-2 and from Ha-CoV-2 was plotted. (E) Rapid quantification of the anti-SARS-CoV-2 activity of Arbidol. HEK293T(ACE2/TMPRSS2) cells were pretreated for 1 hour with Arbidol. Cells were infected with Ha-CoV-2(Luc) in the presence of Arbidol. Viral entry inhibition was quantified by luciferase assay at 5 hours. An MTT cytotoxicity assay of Abidol was also performed on cells (F). (G and H) Ha-CoV-2(Luc) particles bearing the G614 mutation S or the parent D614 S were assembled, and analyzed for the incorporation of S and N in virions. (I) Ha-CoV-2(Luc)(G614) or Ha-CoV-2(D614) was used to infect target cells, and Luc expression was quantified at 5 hours. An equal level of viral particles was used for infection. Assays were performed in triplicates.