Infectivity and Antigenicity of SARS-CoV-2 Strains in Portugal

weijin huang (✉ huangweijin@nifdc.org.cn)

NIFDC: China National Institute for Food and Drug Control <https://orcid.org/0000-0002-4246-8889>

Hai-xin Wang

NIFDC: China National Institute for Food and Drug Control

Li zhang

NIFDC: China National Institute for Food and Drug Control

zi-teng Liang

NIFDC: China National Institute for Food and Drug Control

jian-hui nie

NIFDC: China National Institute for Food and Drug Control

jia-jing wu

NIFDC: China National Institute for Food and Drug Control

qian-qian li

NIFDC: China National Institute for Food and Drug Control

ru-xia ding

NIFDC: China National Institute for Food and Drug Control

yue zhang

NIFDC: China National Institute for Food and Drug Control

guo-qing chen

NIFDC: China National Institute for Food and Drug Control

you-chun Wang

NIFDC: China National Institute for Food and Drug Control

hui-guo Wang

NIFDC: China National Institute for Food and Drug Control

Research Article

Keywords: SARS-CoV-2, Portugal, Infectivity, Antigenicity, Mutation

Posted Date: July 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-681182/v1>

Abstract

The SARS-CoV-2 virus has had a major impact on global human health. During the spread of SARS-CoV-2, weakened host immunity and the use of vaccines with low efficacy may result in the development of more virulent strains or strains with resistance to existing vaccines and antibodies. The prevalence of SARS-CoV-2 mutant strains differs among regions, and this variation may affect the effectiveness of vaccines. In this study, an epidemiological investigation of SARS-CoV-2 in Portugal was performed, and the VSV-ΔG-G* pseudovirus system was used to construct 12 S protein epidemic mutants, D614G, A222V+D614G, B.1.1.7, S477N+D614G, P1162R+D614G+A222V, D839Y+D614G, L176F+D614G, B.1.1.7+L216F, B.1.1.7+M740V, B.1.258, B.1.258+L1063F, and B.1.258+N751Y.

The mutant pseudoviruses were used to infect four susceptible cell lines (i.e., Huh7, hACE2-293T, Vero, and LLC-MK2) and 14 cell lines overexpressing ACE2 from different species. Mutant strains did not show increased infectivity or cross-species transmission. Neutralization activity was evaluated using the newly constructed pseudoviruses, mouse serum, and 11 monoclonal antibodies. The neutralizing activity in immunized mouse serum was not significantly reduced for the mutant strains. Additionally, mutant strains in Portugal showed escape from 9 of 11 monoclonal antibodies. Neutralization resistance was mainly caused by the S477N, N439K, and N501Y mutations in the Spike receptor binding domain. These findings emphasize the importance of SARS-CoV-2 mutation tracking in different regions for epidemic prevention and control.

Introduction

SARS-CoV-2 has seriously endangered human health. Researchers in a wide range of fields, including virology, molecular biology, immunology, and pharmacology, have studied SARS-CoV-2 with the principal aim of preventing its spread. The frequency of SARS-CoV-2 Spike protein mutations is high, and the neutralizing activity of some vaccines and monoclonal antibodies (mAbs) against mutant South African strains is low [1–3]. Mutant strains of the virus in the United Kingdom, the United States, Brazil, South Africa, Portugal, and other regions have attracted widespread attention. However, very few studies have focused on Portugal. In this study, virus strains containing the N501Y mutation in Portugal are traced. Initially discovered in December 2021, UK variants and additional variants spread to Portugal. To further understand the biological activity of SARS-CoV-2 epidemic strains in Portugal, we used a pseudovirus system with vesicular stomatitis virus (VSV) vectors to simulate the circulating viruses for analyses of the effect of mutations on transmission, infectivity, and antigenicity.

Results

Tracking of Spike Protein Amino Acid Polymorphisms in Portugal

Using publicly available epidemiological surveillance data (<https://covidcg.org/?tab=group>), single non-synonymous substitutions in the SARS-CoV-2 Spike protein in Portugal were tracked. Figure 1-A shows

the percentage of virus strains with polymorphisms among all strains in Portugal. In Portugal, the single amino acid substitutions with the highest frequencies were D614G (91.5%), D839Y (12.8%), A222V (12.3%), P1162R (3.5%), S477N (1.6%), and L176F (1.6%). Therefore, we screened six strains with single-site mutation frequencies greater than 1.5% and further investigated combinations of mutations at these sites.

Tracking of Co-mutations in Strains in Portugal

Using epidemiological data (<https://www.epicov.org/epi3/frontend#1bd174>), combined mutations in Portugal and the six most frequent single amino acid substitutions were tracked. The mutant strain B.1.1.7 spread rapidly in the United Kingdom in December and to various European countries, including Portugal. Among mutant strains that appeared in Portugal in December, the UK strain B.1.1.7 accounted for 73.1%; this strain had joint L216F and M740V mutations. At the same time, the variant B.1.258 with B.1.258 + L1063F and B.1.258 + N751Y also appeared. Figure 1-B shows the counts and changes in virus strains with a high frequency of naturally occurring joint mutations in Portugal. From December 31, 2020 to March 3, 2021, D614G was still the most frequent mutation (94.9%), followed by A222V + D614G (24.0%), B.1.1.7 (15.5%), D839Y + D614G (7.3%), P1162R + D614G + A222V (5.2%), S477N + D614G (5.0%), and L176F + D614G (1.3%). The frequencies of the four variants D614G, A222V + D614G, B.1.1.7, S477N + D614G, and P1162R + D614G + A222V showed an increasing trend, among which the increase in B.1.1.7 was second only to D614G. The frequencies of D839Y + D614G and L176F + D614G in Portugal showed a decreasing trend. The counts of the B.1.1.7 + L216F, B.1.1.7 + M740V, B.1.258, B.1.258 + L1063F, and B.1.258 + N751Y variants did not increase.

Infectivity analysis

Infectivity of mutant pseudoviruses in various cells

To evaluate the infectivity of natural variants in Portugal, we infected susceptible cells (Huh7, hACE2, Vero, and LLC-MK2) with 12 mutant pseudoviruses. As shown in Fig. 2-A, infectivity (as evaluated by RLU values) for all pseudoviruses was highest in Huh7 cells, followed by hACE2 cells, while Vero and LLC-MK2 cells had relatively low infectivity. Figure 2-B shows the infectivity of mutants in descending order of the mutation frequency in Portugal. For the four types of cells, the RLU values for D839Y + D614G and B.1.258 were lower than those for D614G, and the infectivity of P1162R + D614G + A222V, L216F + B.1.1.7 was about 2 times higher than that of D614G. On the whole, estimates of infectivity for the 11 natural mutants were similar to the infectivity of D614G (setting a 4-fold increase as the threshold for a significant difference).

Infectivity of different mutant pseudoviruses in cells with receptor overexpression

SARS-CoV-2 infection occurs by S protein binding to the host cell ACE2 receptor, thereby infecting cells. To further evaluate the infectivity of the natural mutant strains in Portugal, we infected HEK-293T cells overexpressing ACE2 receptors with 12 natural mutant strains and evaluated RLU.

First, we constructed 14 cell lines expressing ACE2 receptors of different species. After transient transfection, flow cytometry was used to detect the expression of ACE2 in each cell. We used untreated HEK-293T cells as a negative control. The rate of ACE2 protein expression was 20.8–43.8%, with an average of 32.3%.

We also infected these 14 cells overexpressing ACE2 receptors with 12 natural mutant pseudoviruses. As shown in Fig. 3, infectivity did not differ by more than four-fold among cells overexpressing ACE2 receptors of different species. All pseudoviruses showed higher infectivity in 293T cells overexpressing mouse ACE2 receptor than in other cells. In cells overexpressing rabbit ACE2 and dog ACE2, all pseudoviruses showed generally low infectivity.

Neutralizing activity

Neutralizing antibody detection in serum after immunization

The distribution of the 12 natural mutations in the SARS-CoV-2 S protein in Portugal evaluated in the study is shown in Fig. 4-A. Currently, most monoclonal antibodies (mAb) are directed against the receptor binding domain (RBD) of the SARS-CoV-2 S protein. Therefore, the impact of the N439K, S477N, N501Y, and A570D mutations on mAb protection were evaluated, and the effects of mutations at other sites on transmission and infectivity of the virus should be further studied.

First, we synthesized different segments of SARS-CoV-2 S peptides, including RBD, S1, and S2, and use these peptides as a vaccine to immunize BALB/c mice to obtain post-immunization serum. We also used the SARS-CoV-2 S full-length plasmid as a DNA vaccine to immunize BALB/c mice. We used these sera and 12 pseudoviruses to evaluate protective effects of the neutralizing antibody (Fig. 4-B). Using the neutralization ID₅₀ titers for the D614G pseudovirus and different mouse sera for reference, the neutralization activity was evaluated for the other 11 pseudoviruses. The serum neutralized 11 pseudoviruses with no immune escape (defined as a 4-fold reduction in the ID₅₀ value compared with that for D614G).

Monoclonal antibody neutralization activity

To determine the specific sites affecting the antigenicity of the virus in Portugal, we constructed seven pseudoviruses, S477N, D839Y, L176F, L216F + D614G, M740V + D614G, L1063F + D614G, and N751Y + D614G. All pseudoviruses constructed in this paper were treated with 11 mAbs. The IC₅₀ values for the 11 monoclonal antibodies were similar (i.e., approximately 0.03–0.18 µg/mL), with no significant differences ($P > 0.05$).

As shown in Fig. 5, the epidemic strain S477N + D614G (accounting for 5.0% of strains) and its corresponding single-site mutant in Portugal can escape the monoclonal antibody 09-7B8. Accordingly, escape could be attributed to the S477N mutation (using D614G as the standard and a reduction in neutralizing activity of > 4 times as the threshold for immune escape). We also found that the UK epidemic strain B.1.1.7 and its variants can escape the monoclonal antibodies 03-1F9, 2H10, 03-10D12-

1C3, 03-10F9-1A2, 11D12-1, CB6, and HB27. However, no immune escape was found for M740V + D614G and L216F + D614G. Therefore, escape could mainly be explained by the mutation in B.1.1.7. In addition to escape from the monoclonal antibody HB27 by the UK strain, immune escape was also observed for B.1.258 and its variants. Compared with the neutralizing effects of L1063F + D614G and N751Y + D614G, escape can be explained by the mutation in B.1.258.

Discussion

Coronaviruses have the longest genomes among RNA viruses and are highly prone to mutations. SARS-CoV-2 is a coronavirus and therefore real-time monitoring of the impact of SARS-CoV-2 mutations is essential. The need for biosafety level three laboratories, infection risk for operators, and shortage of experimental equipment have limited the development of SARS-CoV-2 vaccines. In this study, the VSV system was used to construct pseudoviruses harboring high-frequency SARS-CoV-2 mutations circulating in Portugal, including D614G, A222V + D614G, B.1.1.7, D839Y + D614G, P1162R + D614G + A222V, S477N + D614G, and L176F + D614G, as well as mutations in UK strains, e.g., B.1.1.7 + L216F, B.1.1.7 + M740V, B.1.258, B.1.258 + L1063F, and B.1.258 + N751Y. Infectivity and antigenicity were analyzed.

There was no significant difference in infectivity between the high-frequency mutants in Portugal and the D614G mutant pseudovirus. However, the infectivity of P1162R + D614G + A222V was about 2 times greater than that of D614G. The infectivity of D614G + A222V was similar to that of D614G. Therefore, the amino acid change at position 1162 may influence the infectivity of the virus. A previous study has shown that infectivity does not differ significantly between the D614G mutant and B.1.1.7 in cells containing human ACE2 receptors, consistent with the results of this study [4]. In this study, the infectivity of B.1.1.7 was not affected by the addition of the M740V mutation; however, the additional L216F mutation increased infectivity slightly (about 2-fold). We hypothesized that the mutation at position 216 altered infectivity. There was no significant difference between other mutant strains and D614G with respect to infectivity. When we used the newly constructed pseudoviruses to infect cells overexpressing receptors of different species, infectivity was consistently high in cells containing mouse ACE2 receptors, consistent with the results of Gu et al.[5]

During the ongoing pandemic, the emergence of various SARS-CoV-2 mutants has become a major issue. Mutants may have a stronger transmission capacity or the ability to evade neutralizing mAbs and polyclonal antibodies. This may reduce the protective effect of vaccines or neutralizing mAbs developed based on the original genotypes. Nie et al. found that the neutralization effect of the 501 Y.V2 variant is reduced when immunized serum against the South African epidemic strain [4]. We speculate that established vaccines prepared against WuHan-01 may have reduced neutralizing activity against current strains in Portugal. Because the mutation sites in the epidemic strains in Portugal are located in different regions of the spike protein, we used the SARS-CoV-2 spike full-length plasmid and three RBD, S1, and S2 peptides to obtain immunized mice for serum collection. In this analysis, serum neutralizing activity did not differ among pseudoviruses.

Interestingly, the majority of high-frequency mutants in Portugal (i.e., A222V + D614G, B.1.1.7, D839Y + D614G, P1162R + D614G + A222V, S477N + D614G, and L176F + D614G but not B.1.1.7 and S477N + D614G) did not show immune escape from mAbs used in this study was not observed. The antigenicity of B.1.1.7 and the viruses with combined mutations M740V + B.1.1.7, L216F + B.1.1.7, and B.1.258 and the combined mutations B.1.258 + L1063F and B.1.258 + N751Y differed. Immune escape from the mAbs 03-1F9, 2H10, 03-10D12-1C3, 03-10F9-1A2, 11D12-1, CB6, and HB27 was detected. The epitopes 03-10D12-1C3 and CB6 contain the 501 site [6], and other monoclonal antibodies in which escape is observed may explained by this site. The neutralizing activity of various RBD mAbs was significantly reduced. Previous studies have shown that the N501Y mutation affects the antigenicity of the S protein. Since this site is located in the RBD region, most mAbs are directed against the RBD. Therefore, pseudoviruses containing the N501Y mutation will show immune escape or result in a decrease in the protective effect of the mAb [4, 7, 8]. B.1.258 (including N439K and Δ 69-70del) can also cause immune escape from the mAb HB27 [9]. For the mAb 09-7B8, immune escape has been reported for S477N + D614G and the corresponding single mutant S477N. This natural mutation accounts for 5.0% of strains in the Portuguese region. Except for X593 and 05-9G11-1G1, for the remaining nine mAbs evaluated in this study, immune escape by individual mutant strains was observed. To ensure the protective effect of vaccines, cocktail therapies are recommended against SARS-CoV-2 mutant strains. It is also improve to continue to monitor SARS-CoV-2 mutations in different regions in real time and to use these data to adjust epidemic prevention and control measures.

Our laboratory has studied mutant strains in South Africa, the United Kingdom, and Brazil, and this study extends our analysis to mutant strains in Portugal. The results are important for SARS-CoV-2 mutation tracking and provide data for SARS-CoV-2 epidemic prevention and control.

Materials And Methods

Experimental Materials

Reagents

The main reagents, cells, and monoclonal antibodies used in the study are shown in Table 1.

Mouse sera

The mouse serum used in this experiment was maintained in our laboratory. S1, S2, RBD, and S full-length plasmids were used to immunize BALB/c mice to obtain serum for the detection of mutant pseudovirus activity. The BALB/c mice were divided into four groups, namely S1, S2, RBD protein groups (10 mice per group) and the S full-length plasmid group (15 mice). Serum samples from every 5 mice were combined and labeled S1-1, S1-2, S2-1, S2-2, RBD-1, RBD-2, S-1, S-2, and S-3. The animal research protocol was approved by the Animal Welfare Ethical Review Committee of the National Institute of Food and Drug Control.

Experimental methods

Plasmid construction

Mammalian codon optimization was performed on the plasmid expressing SARS-CoV-2 Spike (GenBank: MN908947). It was inserted into the eukaryotic expression vector pcDNA3.1 using the BamHI and XhoI sites and named WuHan-1.

A total of 14 plasmids expressing the ACE2 receptor were constructed, including the ACE2 receptors of human (BAB40370.1), mink (QNC68911.1), dog (MT663955), cat (MT663959), pangolin (XP_017505746.1), pig (NP_001116542.1), rat (ABN80106.1), bat (KC881004.1), cow (NP_001019673.2), rabbit (MT663961), ferret (MT663957), sheep (XP_011961657.1), civet (AY881174.1), and monkey (MT663960). A FLAG tag (GACTACAGAGACGATGATAAG) was inserted at the 3' end. The 14 ACE2 sequences were codon-optimized and synthesized by General Biologicals Co., Ltd. (Taiwan). Each ACE2 sequence was inserted into the eukaryotic expression vector pRP[Exp]-EGFP-CMV using BamHI and XhoI sites to obtain plasmids expressing ACE2 proteins of different species.

Mutant construction

Using the point mutation method, based on the WuHan-1 plasmid, 19 mutants were constructed: D614G, A222V + D614G, B.1.1.7, S477N + D614G, P1162R + D614G + A222V, D839Y + D614G, L176F + D614G, B.1.1.7 + L216F, B.1.1.7 + M740V, B.1.258, B.1.258 + L1063F, B.1.258 + N751Y, S477N, D839Y, L176F, L216F + D614G, M740V + D614G, L1063F + D614G, and N751Y + D614G. The point mutation method was performed as described in our previous study [10, 11]. In short, PCR amplification was performed using the WuHan-1 plasmid as a template. The PCR product was digested with Dpn I (NEB) overnight and used to transform DH5α *E. coli* competent cells. Then, cells were spread on the corresponding plate and incubated overnight at 37°C. A single colony was selected and sequenced to confirm the successful generation of the mutation. Primers corresponding to the mutation sites are shown in Table 2.

Preparation of ACE2-overexpressing cells

Cells expressing ACE2 receptors of different species were prepared. Using T75 flasks, Lipofectamine3000 transfection reagent and 30 µg of receptor plasmid were used to transfect HEK-293T cells, yielding ACE2 receptor-overexpressing cells. The medium was the same as that used for HEK-293T cells. After culturing at 37°C and 5% CO₂ for 24 h, the expression of the labeled ACE2 gene on the cell surface was evaluated by flow cytometry. Approximately 1 × 10⁶ cells/tube were treated with 1 µg/mL PE-labeled anti-labeled antibody (biological preparation).

Preparation of pseudoviruses

SARS-CoV-2 pseudoviruses with spike mutations were constructed using previously described methods [10, 11]. The day before transfection, the concentration of HEK-293T cells was adjusted to 5–7 × 10⁵ cells/mL, followed by incubation overnight at 37°C and 5% CO₂. When the cells reached 70–90%

confluence, the culture medium was aspirated and infected with 15 mL of VSV-ΔG-G* pseudovirus with a TCID₅₀ of 7×10^4 /mL. At the same time, 30 μg of the S protein expression plasmid was transfected with Lipofectamine3000, and the cells were cultured in an incubator at 37°C and 5% CO₂. After 4–6 h, the cell culture medium was discarded and cells were gently washed twice with PBS containing 1% FBS. Next, 15 mL of fresh medium was added to the T75 cell culture flask, which was placed in a 37°C, 5% CO₂ incubator for 24 h. The supernatant (containing SARS-CoV-2 pseudovirus) was then collected.

Quantification of pseudoviruses

Pseudovirus RNA was extracted using the QIAamp Virus RNA Mini Kit (Qiagen, Hilden, Germany). The RT-PCR Kit (Invitrogen) was used to obtain viral DNA by reverse transcription. RT-PCR was performed with TBGreen premixed ExTaqII (Takara). The virus copy number was calculated with the P protein gene plasmid of VSV as the standard.

Infection detection

Cells (3×10^4 /100 μL) were added to each well of a 96-well cell culture plate, followed by the addition of 100 μL of the pseudovirus. After 24 h of incubation at 37°C and 5% CO₂, chemiluminescence was monitored.

The volume of the supernatant in each well was adjusted to 100 μL, followed by the addition of 100 μL/well luciferase substrate and cell lysis buffer (Perkin Elmer, Fremont, CA, USA). After 2 min, 150 μL of the lysate was transferred to an opaque 96-well plate. PerkinElmer Ensign was used to detect the luminescence signal, and data were recorded as relative luminescence units (RLU). The virus copy number calculated by the above quantitative method was used to convert the RLU value to the copy number.

Neutralization test

Luciferase gene expression was evaluated to determine the inhibitory effect of mAbs and serum on pseudovirus entry. The sample was serially diluted by a factor of 3 (initial dilution, 30-fold), with a total of six dilutions, and then 50 μL of the virus solution was added to each well. Each 96-well plate included six virus control wells (no antibody/serum) and six cell control wells (no virus or antibody). The 96-well plate was incubated at 37°C for 1 h, followed by the addition of 3×10^4 /100 μL Huh7 cells to each well. After incubation for 24 h at 37°C and 5% CO₂, luminescence was detected. The Reed-Muench method was used to calculate the sample EC₅₀ (50% effective concentration) [11].

Declarations

Acknowledgments

The authors were supported financially by National Key Research and Development Program of China (No. 2021YFC0863300) .

Declaration of Interests

The authors declare no competing interests.

References

1. Sandile Cele , Inbal Gazy , Laurelle Jackson , et al. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature*. 2021 May;593(7857):142-146.
2. Shen, X, H Tang, R Pajon, et al. Neutralization of SARS-CoV-2 Variants B.1.429 and B.1.351. *N Engl J Med*. 2021 Apr 7; NEJMc2103740. doi: 10.1056/NEJMc2103740. Online ahead of print.
3. Andreano, E, G Piccini, D Licastro, et al. SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. *bioRxiv*. 2020 Dec 28;2020.12.28.424451. doi: 10.1101/2020.12.28.424451. Preprint.
4. Li Q , Nie J , Wu J , et al. SARS-CoV-2 501Y.V2 variants lack higher infectivity but do have immune escape[J].*Cell*. 2021 Apr 29;184(9):2362-2371.e9. DOI: 10.1016/j.cell.2021.02.042. Epub 2021 Feb 23..
5. Gu, H, Q Chen, G Yang, et al. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* 2020; 369(6511): 1603-1607. DOI: 10.1126/science.abc4730.
6. Huang Y , Sun H , Yu H , et al. Neutralizing antibodies against SARS-CoV-2: current understanding, challenge and perspective[J]. *Antibody Therapeutics*, 2020(4):4.
7. Chen, R E, X Zhang, J B Case, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum derived polyclonal antibodies[J]. *Nat Med*. 2021 Apr;27(4):717-726. doi: 10.1038/s41591-021-01294-w.
8. Wang, P, M S Nair, L Liu, et al. Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7[J]. *Nature*. 2021 May;593(7857):130-135. doi: 10.1038/s41586-021-03398-2.
9. Thomson, E C, L E Rosen, J G Shepherd, et al. Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. *Cell* 2021; 184(5): 1171-1187 e20. DOI: 10.1016/j.cell.2021.01.037.
10. Nie, J, Q Li, J Wu, et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerg Microbes Infect* 2020; 9(1): 680-686. DOI: 10.1080/22221751.2020.1743767.
11. Nie, J, Q Li, J Wu, et al. Quantification of SARS-CoV-2 neutralizing antibody by a pseudotyped virus-based assay. *Nat Protoc* 2020; 15(11): 3699-3715. DOI: 10.1038/s41596-020-0394-5.

Tables

Table 1. Cell lines and reagents

Cell lines	Source
HEK-293T	Our laboratory
Huh7	Our laboratory
Vero	Our laboratory
LLC-MK2	Our laboratory
293T-ACE2	Sino Biological
mAb	Source
03-1F9	Beijing Biocytogen Co., Ltd.
09-7B8	Beijing Biocytogen Co., Ltd.
09-4E5-1G2-2H10	Beijing Biocytogen Co., Ltd.
03-10D12-1C3	Beijing Biocytogen Co., Ltd.
03-10F9-1A2	Beijing Biocytogen Co., Ltd.
11D12-1	Beijing Biocytogen Co., Ltd.
05-9G11-1G1	Beijing Biocytogen Co., Ltd.
CB6	Laboratory of Jinghua Yan
X593	Laboratory of Xiaoliang Xie
HB27	Sino Biological
Reagent name	Source
Lipofectamine3000 Transfection Reagent	Invitrogen
Bright-Glo Fluorescence Detection Reagent (substrate)	Promega
PE anti-DYKDDDDK Tag Antibody	Biolegend
Hygromycin B	Gibco

Table 2. Primer sequences

Name	Sequence
D614G-F	TGCTGTACCAGGGCGTGAATTGCACCGAGGT
D614G-R	ACCTCGGTGCAATTCACGCCCTGGTACAGCA
M740V-F	AGCGTGGACTGCACCgtgTACATCTGCGGCGACA
M740V-R	TGTCGCCGCAGATGTAcacGGTGCAGTCCACGCT
L216F-F	TCTGGTGAGAGACTtcCCTCAGGGCTTCAGCGCCCT
L216F-R	AGGGCGCTGAAGCCCTGAGGgaaGTCTCTCACCAGA
N751Y-F	ACCGAGTGCAGCtacCTGCTGCTGCAGTACGG
N751Y-R	CCGTACTGCAGCAGCAGgtaGCTGCACTCGGT
L1063F-F	CGCTCCACATGGCGTGGTGTTCttcCACGTGACCT
L1063F-R	AGGTCACGTGgaaGAACACCACGCCATGTGGAGCG
P1162R-F	AGAATCACACCAGCcgaGACGTGGACCTCGGT
P1162R-R	ACCGAGGTCCACGTctcgGCTGGTGTGATTCT
A222V-F	CCTCAGGGCTTCAGCGTGCTGGAGCCTCTGGTGGA
A222V-R	TCCACCAGAGGCTCCAGCACGCTGAAGCCCTGAGG
D839Y-F	TTCATCAAGCAGTACGGCtatTGCCTAGGTGATA
D839Y-R	TATCACCTAGGCAataGCCGTACTGCTTGATGAA
L176F-F	TACGTGAGCCAGCCTTTTctcATGGACCTGGA
L176F-R	TCCAGGTCCATgaaGAAAGGCTGGCTCACGTA
S477N-F	TACCAGGCCGGAATACACCGTGTAATGGCGTGGA
S477N-R	TCCACGCCATTACACGGTGTATTGCCGGCCTGGTA
A570D-F	CAACAATTCGGCAGAGACATCGACGACACCACAGATGCTGTAAGAGAC
A570D-R	GTCTCTTACAGCATCTGTGGTGTCTCGATGTCTCTGCCGAATTGTTG
D1118H-F	ACGAGCCTCAGATCATCACCACCCACAATACCTTCGTGAGCGGCAA
D1118H-R	TTGCCGCTCACGAAGGTATTGTGGGTGGTGATGATCTGAGGCTCGT
69-70del-F	CGTGACCTGGTTCCACGCCATCAGCGGCACCAATGGCACCAAGAGATTC
69-70del-R	GAATCTCTTGGTGCCATTGGTGCCGCTGATGGCGTGGAACCAGGTCACG
N501Y-F	AGAGCTACGGCTTCCAGCCTACCTACGGCGTGGGCTACCAGCCTTACAG
N501Y-R	CTGTAAGGCTGGTAGCCACGCCGTAGGTAGGCTGGAAGCCGTAGCTCT

P681H-F	CTACCAGACCCAGACCAATAGCCACAGAAGAGCCAGAAGCGTGGCCAGCC
P681H-R	GGCTGGCCACGCTTCTGGCTCTTCTGTGGCTATTGGTCTGGGTCTGGTAG
S982A-F	TACTCAACGACATCCTGGCGAGACTGGACAAGGTGGAGGCCGA
S982A-R	TCGGCCTCCACCTTGTCCAGTCTCGCCAGGATGTCGTTGAGTA
T716I-F	CAATAATAGCATCGCCATCCCTATCAATTTACCATCAGCGTGACCAC
T716I-R	GTGGTCACGCTGATGGTGAAATTGATAGGGATGGCGATGCTATTATTG
145del-F	GACCCTTTCCTGGGTGTTTATCATAAGAACAACAAGAGCTGGATGG
145del-R	CCATCCAGCTCTTGTTGTTCTTATGATAAACACCCAGGAAAGGGTC
N439K-F	CTGCGTGATCGCGTGGAACCTCTAAGAACCTGGACTCGAAAGTTGGAGGC
N439K-R	GCCTCCAACCTTCGAGTCCAGGTTCTTAGAGTTCCACGCGATCACGCAG

Figures

Figure 1

Mutation Tracking in Portugal Note: B.1.1.7 contains S protein mutations, including A570D, D614G, D1118H, H69-V70del, N501Y, P681H, S982A, T716I, and Y145del; B.1.258 contains S protein mutations, including D614G, N439K, and H69-V70del

Figure 2

Infection of four cell types with natural mutant pseudoviruses Notes: (A) A 12 kinds of pseudoviruses respectively infect Huh7, Vero, hACE2, LLC-MK2 four susceptible cells, and detect the RLU value. All results are from three independent experiments (mean $\pm$ SD). The higher the RLU, the higher the infectivity. (B) The infectivity of the mutants was tested in Huh7, Vero, hACE2, LLC-MK2. The RLU value of the mutant infection was measured by a luminometer and compared with the reference strain (D614G). A 4-fold difference is considered significant; this experiment was repeated 6 times (mean $\pm$ SD) .

Figure 3

Pseudovirus infectivity in cells overexpressing ACE2 from different taxa Note: Figure 3-A~C show the infectivity (RLU values) of 12 pseudoviruses against 14 species of ACE2 overexpressing cells and HEK-293T (negative control) cells. The RLU of D614G is used as the standard to compare the remaining 11 pseudoviruses RLU value. The higher the ratio, the stronger the infectivity. When Y=4, it means that the

infectivity is increased by 4 times; when $Y=0.25$, it means that the infectivity is reduced by 4 times. The experiment was repeated 4-6 times (mean $\pm$ SD).

Figure 4

Neutralization activity of mouse serum with different pseudoviruses Notes: (A) Distribution of SARS-CoV-2 mutation sites (B) RBD1 and RBD2 represent the two groups of mouse serum immunized with the peptides of SARS-CoV-2 RBD; S1-1 and S1-2 represent the two groups of mice immunized with the peptides of SARS-CoV-2 S1; S2-1, S2-2 represent the two groups of mouse serum immunized with the SARS-CoV-2 S2; S1, S2, S3 represent three groups of mouse serum immunized with the SARS-CoV-2 S

full-length DNA plasmid. X=0.25 represents a 4-fold decrease in neutralization activity compared with D614G, and X=4 represents a 4-fold increase in neutralization activity.

Figure 5

Neutralization effect of mAbs for different pseudoviruses Note: The neutralization ID50 value for D614G was used as the standard, and neutralization ID50 values were calculated for different mutant strains of pseudoviruses and 11 mAbs. A significant difference was defined as a 4-fold difference from the standard. Blue represents a decrease in neutralizing activity, and red represents an increase in activity.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.doc](#)
- [TableS2.doc](#)