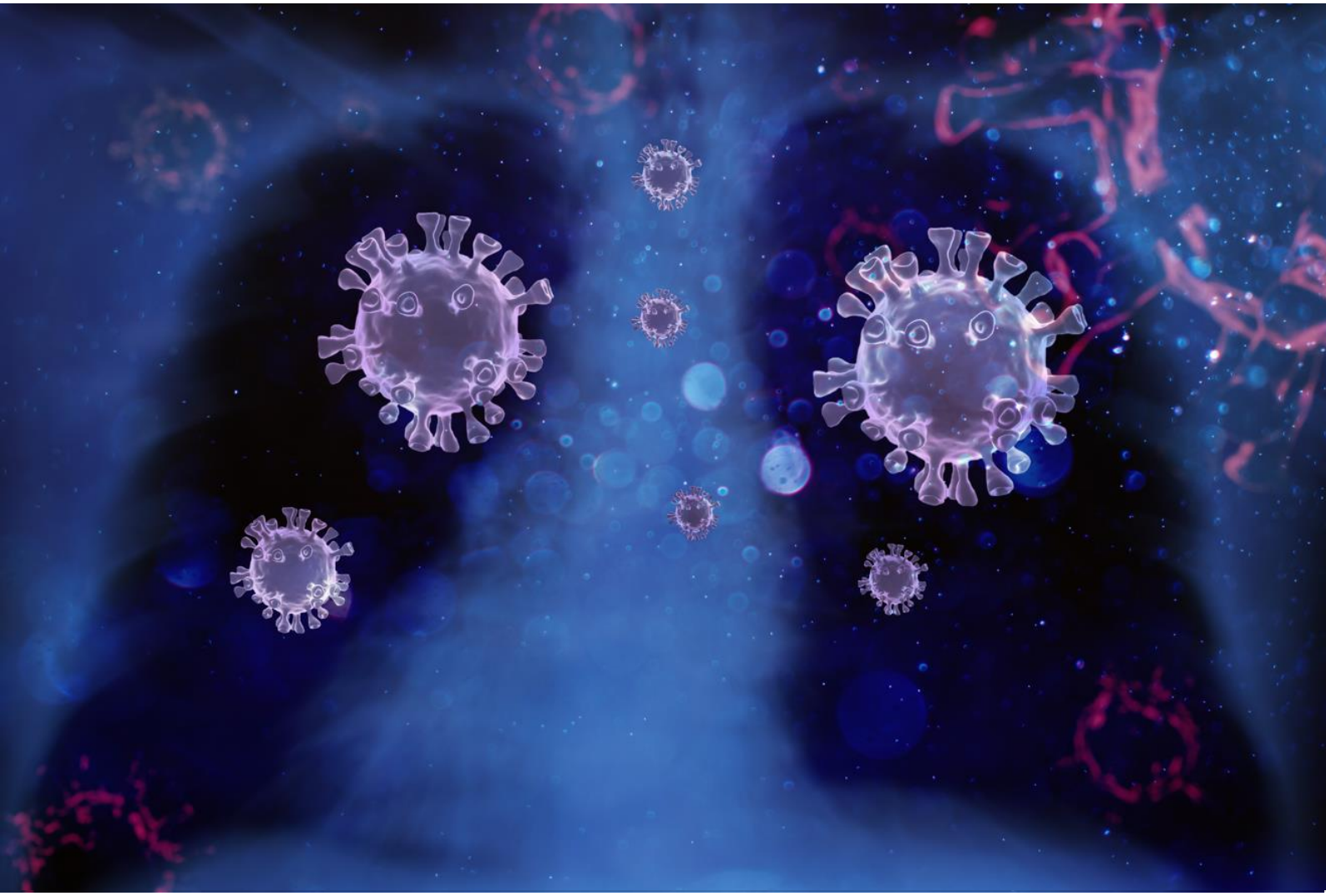
SARS-CoV-2 Pseudovirus Neutralization Assay



Mutant SARS-CoV-2 Pseudotyped Lentiviruses Case Study

Creative Diagnostics offer a range of recombinant proteins, assay kits, antibodies to help accelerate the search for COVID-19 drugs and vaccines.

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Content

Background

SARS-CoV-2 pseudotyped lentivirus is replication-incompetent with safe (BSL-2), easy, and high-throughput viral infectivity and can be used in neutralization assays with standard detection instrumentation. It displays an antigenically correct spike protein on a heterologous virus core and carries a modified genome that expresses a convenient optical reporter gene (GFP or luciferase).

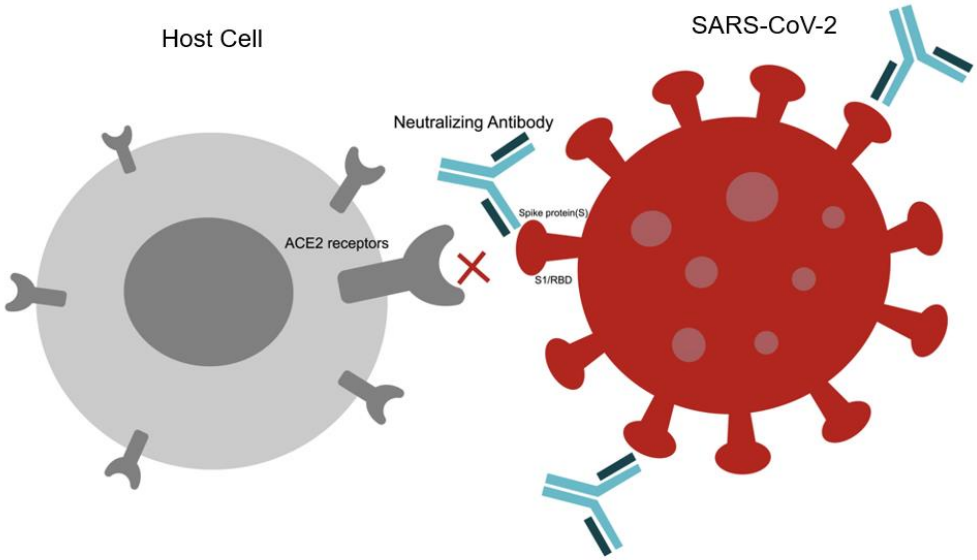


Fig1. Pseudotype-Based Neutralization Assays Principle

Creative Diagnostics has launched a full range of ready-to-use wild-type and newly emerged mutant SARS-CoV-2 pseudotyped lentiviruses to study the interaction between SARS-CoV-2 spike protein and human ACE2 in a physiologically relevant context.

Procedures

Neutralizing serum incubation with pseudovirus | **Thawing, diluting, and plating pseudovirus** | **Seeding and infection of hsACE2/293T cells** | **Quantification**

Neutralizing serum incubation with pseudovirus

- Prepare dilutions of serum sample at 2X the desired final concentration in Cell Culture media. Leave a virus control (VC) without serum - that will be the control for 100% infectivity. Set a positive control with SARS-CoV-2 positive control antibody with neutralization response to SARS-CoV-2 WT and variants. Set a negative control with negative control antibody without neutralization response to SARS-CoV-2.
- Prepare a total volume of the 2X diluted serum of 50 µL/well for a 96-well plate

Thawing, diluting, and plating pseudovirus

- Remove an aliquot of frozen pseudovirus, place in a 4°C ice bath until just thawed.
- Gently mix the pseudovirus tube by inversion.

- Dilute the 20 uL of pseudovirus per well in cell culture media for a total volume of 50 µL/well for a 96-well plate.
- Mix the pseudovirus with the serum dilutions. This will be a total volume of 100 µL/well for a 96-well plate, with the serum and pseudovirus now each at 1X concentration.
- Incubate at 37°C for 1 h

Seeding and infection of hsACE2/293T cells

- Lift a sub-confluent flask of hsACE2/293T cells using 0.05% Trypsin-EDTA, stop trypsinization by adding cell culture media (twice the volume of the Trypsin-EDTA added), pellet the cells by centrifuging in a conical tube for 5 min at 200 g, and resuspend in cell culture media.
- Determine cell density using a hemocytometer or equivalent.
- Infect cells: For a 96 well plate, resuspend cells at a density of 4 x 105 cells/mL. Add 100 µL of the cell suspension (4 x 104 cells) to each well containing the pseudovirus and mix gently by pipetting 1-2 times (see Note 3). Leave a cell control (CC) without pseudovirus.
- Place plate in a 5% CO2, 37°C humidified incubator for 48-72 hours.

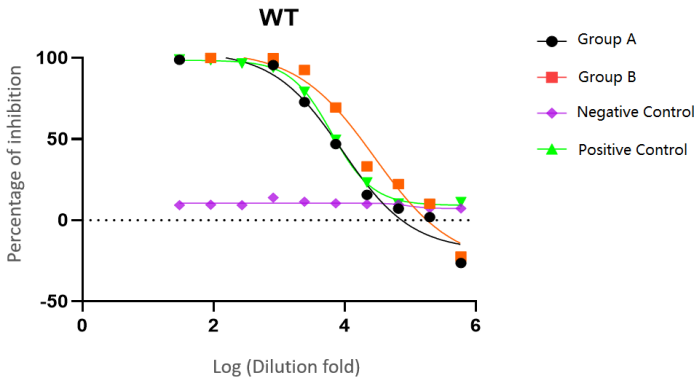
Quantification

- Luciferase activity, expressed in relative light units (RLUs), was determined according to the Luciferase Assay System user's manual.

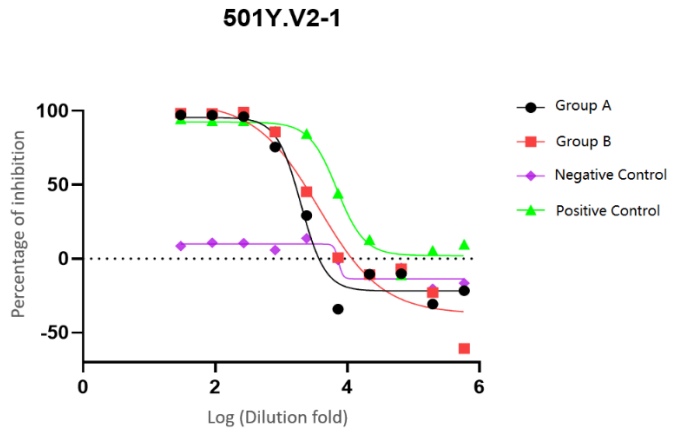
Results

Inhibition curve was performed with convalescent plasma at serial dilutions starting from 1:30 to 1:590490. When the neutralization curve crosses the 50% line, the EC50 for this sample can be calculated. The EC50 was calculated by using the Reed-Muench method automatically in the macro.

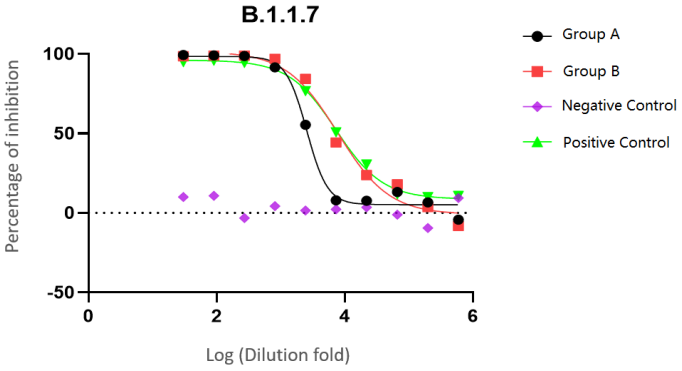
Note: percentage of inhibition（%）=1- (Sample RLU_AVE - Cell Control RLU_AVE) / (Virus Control RLU_AVE - Cell Control RLU_AVE)



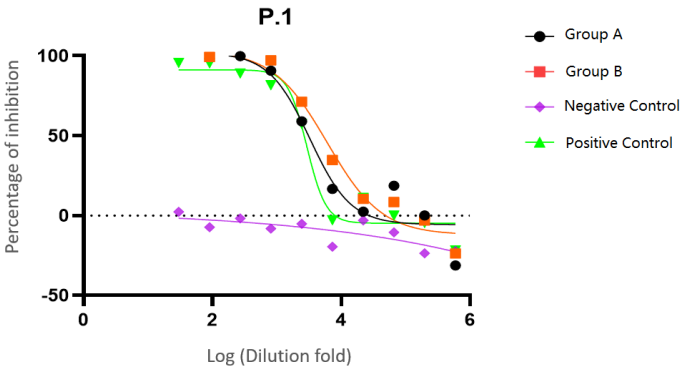
WT	Group A	Group B	Positive Control
EC50	9117	28559	6278
R^2	0.9719	0.9643	0.9702



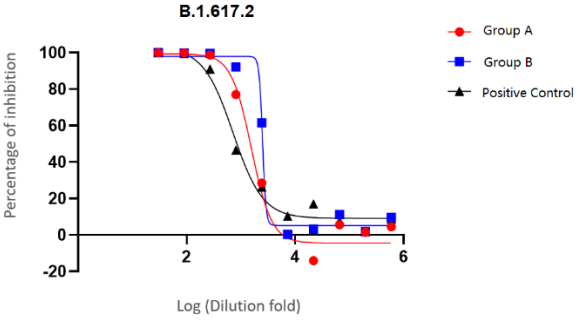
501Y.V2-1	Group A	Group B	Positive Control
EC50	1992	3415	6947
R^2	0.953	0.912	0.966



B.1.1.7	Group A	Group B	Positive Control
EC50	2540	7604	7222
R^2	0.9766	0.9582	0.9606



P.1	Group A	Group B	Positive Control
EC50	3257	5992	2921
R^2	0.9299	0.9436	0.9491



B.1.617.2	Group A	Group B	Positive Control
EC50	1563	~ 2523	718.3
R^2	0.928	0.9702	0.9514

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Contact Us

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