08.03 - ASU BSL3 Lab Results

- Arizona State University Biodesign Institute
- Southwest College Of Naturopathic Medicine & Health Sciences
- DR. Jeffrey Langland Research Director
- February 23, 2021

Project Name	Anti-SARS-CoV-2 properties of novel hand sanitizer solutions
Project Description	Characterization of the long-term anti-SAR-CoV-2 properties of novel hand sanitizer solutions
Project Lead	Southwest College of Naturopathic Medicine, Ric Scalzo Institute for Botanical Research. Dr. Jeffrey Langland, Research Director
Start Date	February 2021
Summary Date	February 23, 2021

Purpose
Characterize the long-term antimicrobial properties of Meditizer^{tm} Hand Sanitizer & Mask Spray products against SARS-CoV-2. Herein referenced as 2 in 1 Hand & Mask Spray.

SARS-CoV2 Long-term killing assay:

Materials:

SARS-CoV-2 strain USA-WA1/2020 (BEI Resources)
Vero E6 cells (ATCC)
BSL3 facility (contract service with Arizona State University, Biodesign Institute)
D-MEM media with 10% fetal bovine serum (COMPLETE MEDIA)
PBS (phosphate buffered saline)

Stocks:

Vero cells were maintained in D-MEM media with 10% fetal bovine serum
All cells were used under limited passage conditions

SARS-CoV-2 virus stocks were grown in Vero cells under standard protocols. Viral titers were determined by plaque assay in Vero cells. Final stock titer was $3x10^7$ PFU/ml

Experimental procedure 1:

1. In the BSL2 tissue culture room, treat four 6-well tissue culture plates with 50 ul of each of the following solutions. Spread solution evenly with the large end of a sterile pipet tip.
    a. PBS
    b. 0.95 Glycerin solution

c. 2 in 1 Invisible Glove
d. Mask Spray

2. Remove the lid and air dry in the hood for 50 min. Cover and immediately bring into the BSL3 facility
3. Dilute SARS-CoV-2 virus stock to 10^5 PFU diluted into 100 ul with PBS
4. For TWO of the six-well plates that have dried for 1 hour, immediately add 100 ul virus solution per well and spread over the surface by rocking. Rock every 5 min.
5. After 25 minutes, add 0.4 ml complete media to each well, pipet/wash over the well 5-times, and transfer the solution to a sterile tube for subsequent titering.
6. For the remaining TWO plates, at 4 hours (1 hour drying + 3 hours dish sitting in the hood), repeat steps 4-5.
7. For the 24 samples total (6 in duplicate at 1 hr, and 6 in duplicate at 4 hr), perform serial dilutions (undiluted, 1:10, 1:100, 1:1000 in complete media). Titer each virus sample by plaque assay on Vero cells by standard protocols

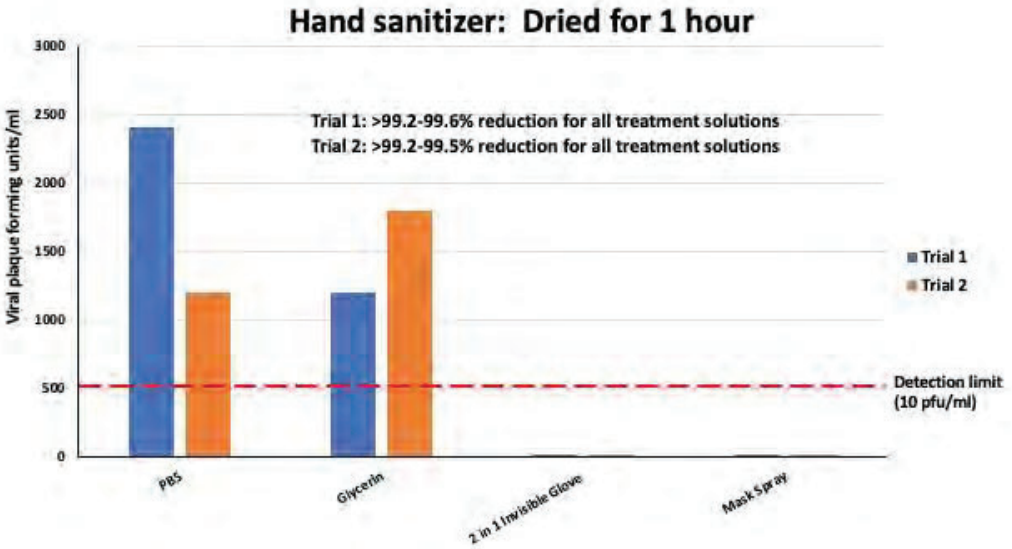
Experimental procedure 2:

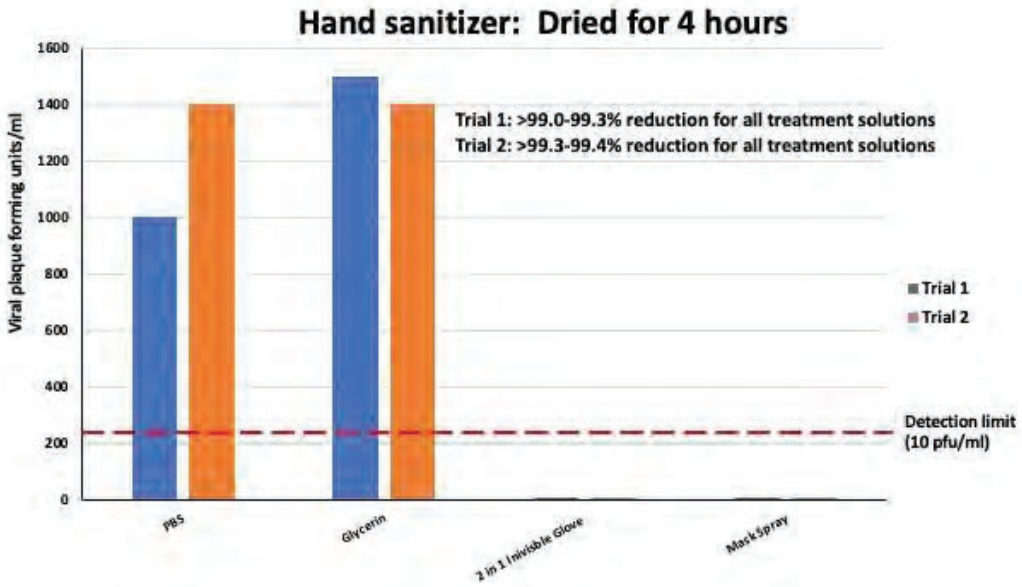
1. In the BSL2 tissue culture room, treat two sets of 6-well tissue culture plates with 50 ul of each of the following solutions. Spread solution evenly with the large end of a sterile pipet tip.
    a. PBS
    b. 0.95 Glycerin solution
    c. 2 in 1 Invisible Glove
    d. Mask spray
    e. 2 in 1 Invisible Glove (diluted 1:5 in PBS)
    f. Mask Spray (diluted 1:5 in PBS)

2. Remove the lid and air dry in the hood for 50 min. Cover and immediately bring into the BSL3 facility
3. Dilute SARS-CoV-2 virus stock to 10^5 PFU diluted into 100 ul with PBS
4. For TWO of the six-well plates that have dried for 1 hour, immediately add 100 ul virus solution per well and spread over the surface by rocking. Rock every 5 min.
5. After 25 minutes, add 0.4 ml complete media to each well, pipet/wash over the well 5-times, and transfer the solution to a sterile tube for subsequent titering.
6. For the remaining TWO plates, at 4 hours (1 hour drying + 3 hours dish sitting in the hood), repeat steps 4-5.
7. For the 20 samples total (10 for the 1 hr, and 10 for the 4 hr), perform serial dilutions (undiluted, 1:10, 1:100, 1:1000 in complete media). Titer each virus sample by plaque assay on Vero cells by standard protocols

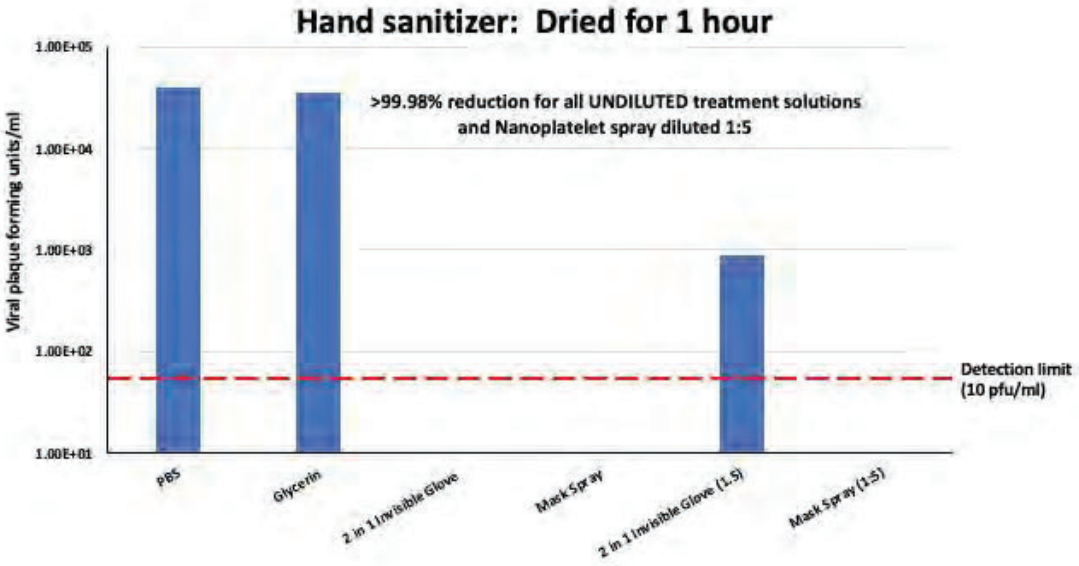
Project results:

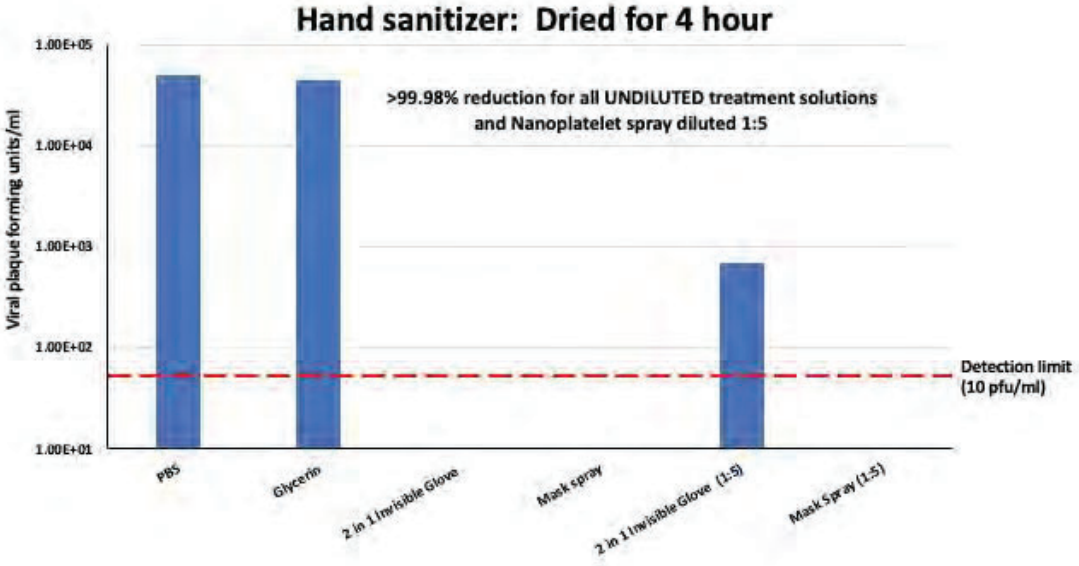
Experimental study 1:





Experimental study 2:


Hand sanitizer: Dried for 1 hour
Viral plaque forming units/ml
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
>99.98% reduction for all UNDILUTED treatment solutions and Nanoplatelet spray diluted 1:5
Detection limit (10 pfu/ml)
PBS
Glycerin
2 in 1 Invisible Glove
Mask Spray
2 in 1 Invisible Glove (1:5)
Mask Spray (1:5)


Hand sanitizer: Dried for 4 hour
Viral plaque forming units/ml
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
>99.98% reduction for all UNDILUTED treatment solutions and Nanoplatelet spray diluted 1:5
Detection limit (10 pfu/ml)
PBS
Glycerin
2 in 1 Invisible Glove
Mask spray
2 in 1 Invisible Glove (1:5)
Mask Spray (1:5)

Results summary:

The results of this study support that the hand sanitizer solutions containing 2 in 1 Hand and Mask Spray are all able to kill the SARS-CoV-2 virus even after drying on a surface for 1 hour or 4 hours.

In the first study, negative control samples (surface treated with PBS or glycerin) had a non-inhibitory effect on the virus with the presence of approximately 1000-2500 virus/ml. When this amount of virus was applied to surfaces treated with any of the hand sanitizer solutions, no virus could be detected in the samples. The detection limit of this assay was 10 virus/ml. Similar results were observed when the hand sanitizer treatments were left on the surface for 1 hour or 4 hours, indicating no loss of virus killing activity over this time period. The results support that these hand sanitizer solutions remain active related to anti-SARS-CoV-2 activity for up to 4 hours on a surface and provide over a 99.0% virus killing response.

In the second study, negative control samples (surface treated with PBS or glycerin) had a non-inhibitory effect on the virus with the presence of approximately 30,000 virus/ml. When this amount of virus was applied to surfaces treated with any of the undiluted hand sanitizer solutions, no virus could be detected in the samples. The detection limit of this assay was 10 virus/ml. Similar results were observed when the hand sanitizer treatments were left on the surface for 1 hour or 4 hours, indicating no loss of virus killing activity over this time period. The results support that these hand sanitizer solutions remain active related to anti-SARS-CoV-2 activity for up to 4 hours on a surface and provide over a 99.98% virus killing response.

In the second study, when the hand sanitizer solutions were diluted 1:5 in PBS and then applied to the surface, virus killing was approximately 90-95% for the hand sanitizer solution containing 2 in 1 Invisible Glove. The Mask Spray diluted 1:5 in PBS was still able to kill with over a 99.98% virus killing response.

Certification:

Experimental design and analysis were conducted at the Southwest College of Naturopathic Medicine, Ric Scalzo Institute for Botanical Research under the guidance and supervision of Dr. Jeffrey Langland, Research Director. Experimental procedures were performed at the Arizona State University Biodesign Institute, Biosafety Level 3 facility.

Results are certified as valid based on experimental procedures performed

Jeffrey Langland
Research Director
Southwest College of Naturopathic Medicine
Ric Scalzo Institute for Botanical Research

SCNM
SOUTHWEST COLLEGE OF NATUROPATHIC MEDICINE & HEALTH SCIENCES