Biomedical Research Laboratory at the
National Center for Biodefense and Infectious Disease
Manassas, Virginia

Study Title

Determination of the Efficacy of Tygrus LLC test substance, Tydracide™ against COVID-19 Virus

Study Performed By:

Dr. Aarthi Narayan and her team at the George Mason University Certified Bio Safety Level 3 Laboratory (BSL3)

Study Coordinated By:

Dr. Anthony Atala, G. Link Professor and Director of the Wake Forest Institute for Regenerative Medicine at Wake Forest University in Winston Salem, North Carolina.

Test Facility

The Biomedical Research Laboratory, National Center for Biodefense and Infectious Disease

Dates of Study:

April 13th – 17th, 2020

Study Purpose

SARS-CoV-2 Viral Assay after treatment five (5) dilutions of Tydracide test compound

Assess the Antiviral Activity of Tydracide Against SARS-CoV-2

Tydracide is a new acid material that has demonstrated virucidal properties with no or minimal toxicity. This SARS-COV-2 Assay, on the virus, which causes COVID-19, is an important step in measuring the efficacy of this new product against the target virus.

Dr. Aarthi Narayan

Dr. Narayan's leads a team at the Biomedical Research Laboratory, National Center for Biodefense and Infectious Disease in Manassas, Virginia, at George Mason University, which is a certified Bio Safety Level 3 Laboratory (BSL3).

The major focus of Dr. Narayan's work is to understand host responses to infection by human viruses. In addition to identifying novel therapeutic candidates, her interests also extend to defining the mechanistic basis behind pathogen inhibition when using host-based therapeutics. Such information can also be utilized to discover novel disease/pathogen specific biomarkers, vaccine and therapeutic candidates. Dr. Narayanan has authored more than 70 manuscripts to date and over 40 are in the context of acute and emerging viral infections. Dr. Narayanan serves as an editor for PLOS One and as PI/Co-PI on several federal and nonfederal awards.

Dr. Anthony Atala

As director of the Institute for Regenerative Medicine, Dr. Atala oversees a team of more than 400 researchers who are dedicated to developing therapies for patients. The Institute houses an FDA compliant GMP facility for the manufacturing of products for human use or exposure, and is able to deliver therapeutics, and direct clinical trials.

Dr. Atala is a recipient of many awards, including the congressionally funded Christopher Columbus Foundation Award, which is bestowed on a living American who is currently working on a discovery that will significantly affect society. In 2011, he was elected to the Institute of

Medicine of the National Academy of Sciences and was inducted to the National Academy of Inventors as a Charter Fellow in 2014. He is the Editor-in-Chief of 3 journals and over 20 books.

History of the Lab - The Biomedical Research Laboratory, National Center for Biodefense and Infectious Disease

The Biomedical Research Lab (BRL) is one of thirteen Regional Biocontainment Laboratories constructed with funding support from the National Institute of Allergy and Infectious Diseases/National Institutes of Health (NIAID/NIH). The BRL is a state-of-the-art laboratory where scientists are performing pioneering research of infectious diseases, both emerging and potential bio threat agents.

The BRL supports research programs of the National Center for Biodefense and Infectious Diseases (NCBID) focusing on host-pathogen interactions using proteomics and nanotechnology as they are applied to diagnostic, therapeutic, and vaccine development. Additionally, the BRL is capable of housing multiple species, from rodents to nonhuman primates.

Scientific Qualifications of Lab:

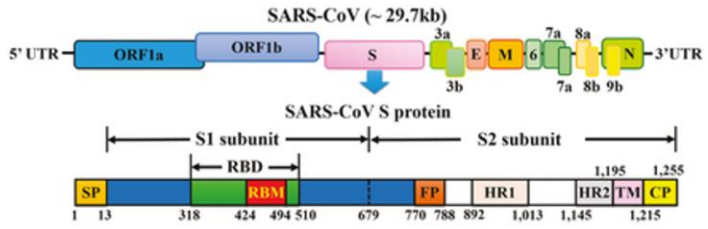
The facility is fully approved and licensed for work by the Center for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture (USDA). The BRL is also fully accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care).

SARS-CoV Virus Information

Coronaviruses constitute the subfamily *Orthocoronavirinae*, in the family *Coronaviridae*, order *Nidovirales*, and realm *Riboviria*. They are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry. The genome size of coronaviruses is one of the largest among RNA viruses. They have characteristic club-shaped spikes that project from their surface.

The genome organization for a coronavirus is 5'-leader-UTR-replicase/transcriptase-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3'UTR-poly (A) tail. The open reading frames 1a and 1b, which occupy the first two-thirds of the genome, encode the replicase/transcriptase polyprotein. The replicase/transcriptase polyprotein self cleaves to form nonstructural proteins. The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid. Interspersed between these reading frames are the reading frames for the accessory proteins.

Figure. Schematic representation of the genome organization and functional domains of S protein for SARS-CoV



Study Methods

50 µL of each Tydracide formula was added to 50 µL of SARS-CoV-2 containing media, titered at 3.5E5. The solution was allowed to incubate for the selected time periods of 1 minutes and 5 minutes. After incubation, a serial dilution was performed in Dulbecco's Modified Eagle's Medium (DMEM) out to 10^{-4} and each dilution was screened using a viral plaque assay.

Twelve (12)-well plates containing VERO cells, a lineage isolated from kidney epithelial cells extracted from African green monkey, had been seeded for 24 hours. Media was aspirated from the wells and 200 µL of each final dilution was added to the well. Plates were allowed to incubate in the solution for one hour with mixing every 15 minutes.

Following incubation, wells were covered with agar-containing media. Plates were returned to the incubator for 48 hours. Plates were then removed and fixed in 10% polymeric formaldehyde (PFA) for 1 hour. PFA was aspirated and the agar plug was removed. Crystal violet was used to visualize any viral plaques that had formed. Plaque counts were recorded, and the data was analyzed.

Test Results:

All final concentrations of Tydracide demonstrated potent antiviral activity. Each sample tested at over a 5-log reduction (>99.999) in SRA-CoV-2 survival at 1 (Table 1) and 5 (Table 2) minutes contact times.

The control Saline Solution showed no significant impact on SARS-CoV-2 survival, as measured as plaque forming units per ml (PFU/ml), after 1 minute and 5 minutes, of exposure times.

Sample one at a 2.5% concentration compound showed no SARS-CoV-2 survival, as measured as plaque forming units per ml (PFU/ml), after 1 minute and 5 minutes of exposure time, with over a 5 log reduction at each time point.

Sample two at a 5.0% concentration compound showed no SARS-CoV-2 survival, as measured as plaque forming units per ml (PFU/ml), after 1 minute and 5 minutes of exposure time, with over a 5 log reduction at each time point.

Sample three at a 15% concentration compound showed no SARS-CoV-2 survival, as measured as plaque forming units per ml (PFU/ml), after 1 minute and 5 minutes of exposure time, with over a 5 log reduction at each time point.

Sample four at a 20% concentration compound showed no SARS-CoV-2 survival, as measured as plaque forming units per ml (PFU/ml), after 1 minute and 5 minutes of exposure time, with over a 5 log reduction at each time point.

Sample five at a 25% concentration compound showed no SARS-CoV-2 survival, as measured as plaque forming units per ml (PFU/ml), after 1 minute and 5 minutes of exposure time, with over a 5 log reduction at each time point.

Tydracide Test Results against SARS-CoV-2

1:1 Tydracide Against SARS-CoV-2	Control Sample	Sample One	Sample Two	Sample Three	Sample Four	Sample Five
Concentration		2.50%	5.00%	15.00%	20.00%	25.00%
One Minute	1.10E +05	N/D	N/D	N/D	N/D	N/D
Log Reduction		> 5 log	> 5 log	> 5 log	> 5 log	> 5 log
Five Minutes	1.10E +05	N/D	N/D	N/D	N/D	N/D
Log Reduction		> 5 log	> 5 log	> 5 log	> 5 log	> 5 log

Definition: N/D – non-detectable

Conclusion

All Samples of Tydracide at final concentrations from 2.5% up to 25% showed effectiveness against the Coronavirus, SARS-CoV-2 after 1 minute and 5 minutes of exposure; with over a 5 log reduction in each instance in plaque forming units per ml (PFU/ml), with no detectable survival. Using this widely accepted antiviral suspension test protocol; the test demonstrated that even the lowest concentration of 2.5% Tydracide was able to completely eliminate Coronavirus, SARS-CoV-2 after just a one-minute contact time. The results of these benchmark studies demonstrate that Tydracide has potential for use as an extremely safe and highly effective disinfectant to combat the COVID-19 virus.

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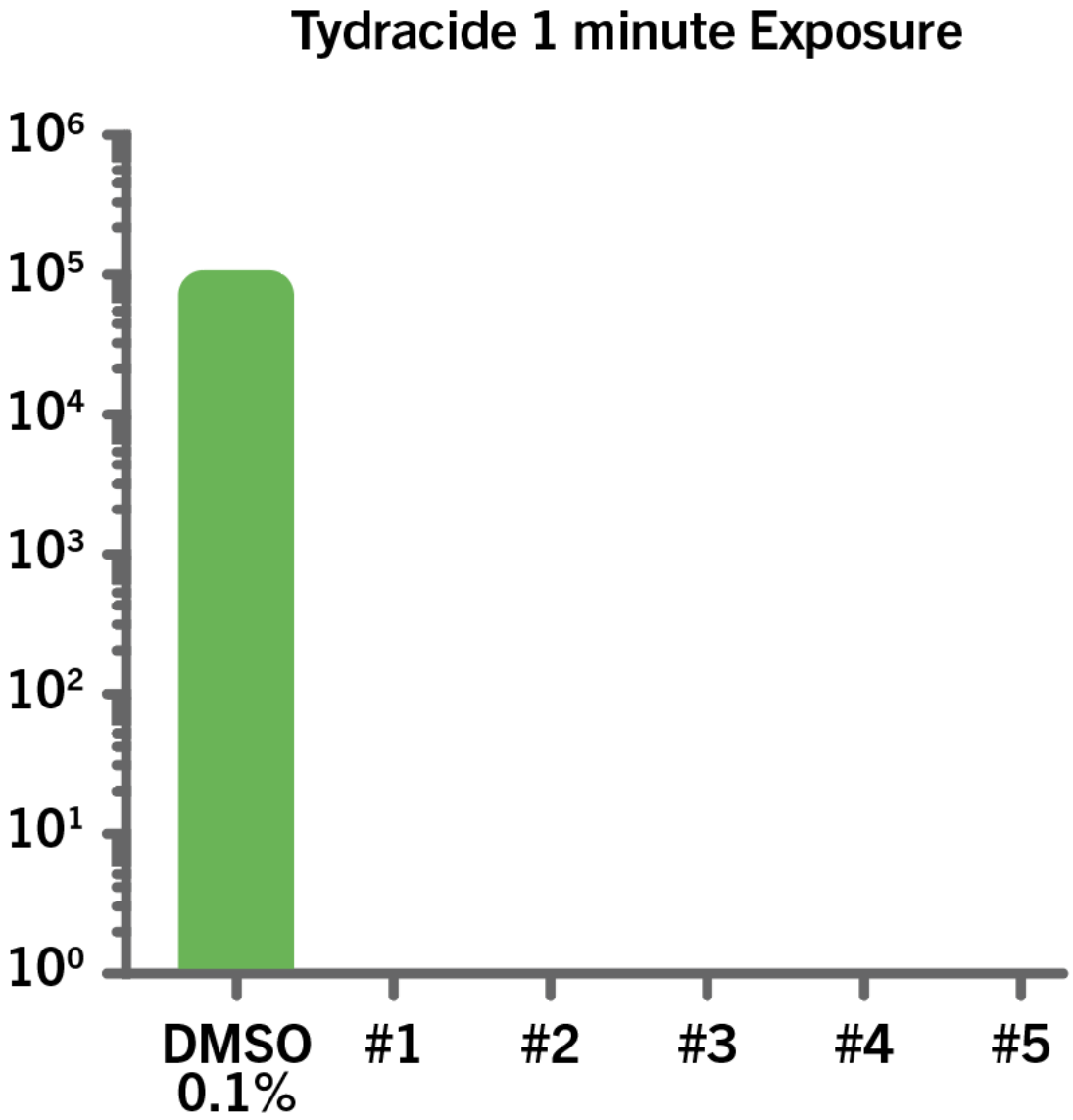
Anthony Atala, MD, Study Coordinator


Tydracide 1 minute Exposure
10⁶
10⁵
10⁴
10³
10²
10¹
10⁰
DMSO 0.1%
#1
#2
#3
#4
#5