A Review of the Synexis Dry Hydrogen Peroxide System for use Against the Novel SARS-CoV-2 Pandemic Virus

Compiled by Synexis Biodefense

Summary

The current SARS-CoV-2 (coronavirus) caused COVID-19 pandemic has forced the world to respond in dramatic fashion in an effort to stem transmission and reduce incidence of disease. To aid in this effort, the United States EPA has released guidelines for sanitation products to make claims of efficacy against the novel SARS-CoV-2. The EPA writes: "***if an antimicrobial product can kill a small, non-enveloped virus it should be able to kill any large, non-enveloped virus or any enveloped virus."*** Herein we review multiple experiments where dry hydrogen peroxide (DHP) from Synexis Biodefense was tested against multiple small, non-enveloped viruses as well as enveloped viruses, including a gammacoronavirus. In all cases, DHP significantly reduced viral load on surfaces and in the air of the test area in as little as 60 minutes. This data shows that DHP may be used to combat the novel SARS-CoV-2 pandemic currently devastating the world.

Introduction

Viruses are the most common form of biological entity on the planet and, in some cases, are considered the most basic form of life. Viruses predominantly "live" inside host cells, where replication and new virion production occurs. Some viruses can maintain themselves in a dormant state outside the host, but are most susceptible to destruction from environmental factors or human interventions at this point. Viruses infect all types of life, many causing disease, and affect almost all systems, including respiratory, enteric, and immune. The major route of transmission for viruses differs based on the main point of infection, i.e. respiratory viruses through aerosols, enteric viruses through the fecal/oral route, etc., but all viruses can be transmitted through contact with contaminated surfaces. Infectivity of a virus from contact with a contaminated surface will vary by virus, but is a substantial and often overlooked route for viral spread.

Respiratory viruses are common in the human population and symptoms from infection with this class varies widely, from no apparent signs to the common cold to pandemics leading to significant morbidity and mortality. Most of the human population experiences cold-like symptoms caused by infection with a respiratory virus every year. Common respiratory viruses include Influenza, Coronavirus (CoV), Rhinovirus, and Respiratory Syncytial Virus (RSV), along with many others. Most of the population is acutely aware of influenza and we are encouraged to get vaccinated against it each year. But most people are not as acutely aware of other respiratory viruses like Coronavirus, even though they cause approximately a third of common colds (1). In fact, most people associate the common cold with flu and Coronaviruses rarely ever get mentioned, though human Coronaviruses 229E, NL63, OC43, and HKU1 are very common.

One reason these CoVs do not get recognized is that they cause inapparent or very mild infections, and medical care is not needed for these viruses.

There have been more severe outbreaks of CoVs in the past, namely SARS-CoV (Severe Acute Respiratory Syndrome) and MERS-CoV (Middle East Respiratory Syndrome), but those viruses originated and were most prevalent in China and Middle Eastern countries, respectively, and did not transmit efficiently human to human. Consequently, the virus spread was minimal and did not gain international recognition in the same way Influenza viruses (such as the 1918 Spanish Flu or the 2009 Swine Flu) have. Both SARS-CoV and MERS-CoV were viruses that transmitted from animal hosts to humans, making them zoonotic, which is a common transmission mechanism. In late fall of 2019, another CoV made the jump from animals to humans in China and has been named SARS-CoV-2 due to the similarity in disease presentation to the original SARS-CoV (2). This virus differs from the original in that it is much more efficient at human to human transmission than the previous CoV outbreaks (3), and subsequently spread from China to nearly every part of the world. It also seems to cause a more severe disease, named COVID-19 (Coronavirus disease 2019), than the original SARS-CoV (3). At the time of this writing (March 26, 2020), there are 511,603 confirmed cases with 23,568 total deaths worldwide, for a mortality rate of 4.61% (Johns Hopkins COVID 19 Resource Center). It should be noted that the mortality rate is most likely much lower because the infection rate may be up to 10 times higher than reported, due to many infections causing no symptoms (which won't lead to testing) and a general unavailability of tests required for confirmation of infection.

Control of the current COVID-19 pandemic is difficult as this virus is antigenically different from anything we have seen and therefore we have no population immunity. As more people are infected, immunity will build, but so will mortality in sensitive populations. Vaccines are very effective at controlling CoV infections in general, but it often takes years to develop effective vaccines against this class of viruses. Indeed, our best intervention for overcoming this pandemic is reducing the number of hosts available for infection through social distancing, sheltering-in-place, general hygiene (hand washing, using hand sanitizer, not touching your face), and cleaning and disinfection. Most sanitizers are effective at destroying enveloped viruses such as CoVs, which, as a class, are more susceptible to sanitizing agents than small or large non-enveloped viruses. Thorough sanitation requires proper application and contact time with all places, including surfaces where viruses may remain intact for some time.

Principle of Synexis' Dry Hydrogen Peroxide

Typical cleaning and disinfection require proper application, concentration, and contact time which can be very effective if done well. However, it is only a point-in-time cleaning and re-contamination can occur. This is why we are encouraged to wash our hands or use hand sanitizer after touching anything. And with what is known about the SARS-CoV-2 virus maintaining itself on varied surfaces for up to 3 days (4) (with genomic material being detected on cruise ships for

up to 17 days post evacuation but before cleaning (CDC Website)), a method for destruction of viruses that is continually active would be beneficial.

Synexis Biodefense Systems technology produces a completely non-aqueous form of hydrogen peroxide in the same physical state as the oxygen and nitrogen in the air – dry hydrogen peroxide (DHP) – for the reduction of pathogens in occupied spaces. As an active, full-time countermeasure to the active, full-time challenge of microbial threats, DHP technology achieves something that intermittent disinfection technologies cannot: the sustained reduction in airborne and surface-contaminating microbes over broad areas.

DHP is generated on-site from humidity and oxygen in the ambient air and becomes part of the air. Operating at concentrations well below those naturally maintained by enzymes in the human lung, and less than 1/25th of the OSHA safety limit, DHP diffuses into hard-to-reach spaces that act as contamination reservoirs.

A critical differentiator for DHP from other forms of hydrogen peroxide utilized for whole room microbial reduction is the fact that DHP can be safely delivered in occupied spaces; therefore, it does not negatively impact workflow or patient throughput.

Standard of Disinfection

As the SARS-CoV-2/COVID-19 pandemic is currently ongoing, and with many research facilities around the world shuttered in an effort to stem viral spread or transitioned to diagnostic capability for virus detection, it is difficult to test any active agent against the virus for the time being. Nevertheless, much is known about CoVs and their response to common sanitizers and disinfectants, and information can be gleaned from previous research with those viruses or others like them. In fact, The Environmental Protection Agency (EPA) has established a policy which enables the makers of registered hospital/healthcare or broad-spectrum disinfectant products to make limited claims of efficacy against 2019 Novel Coronavirus [Emerging Viral Pathogen Program Guidance (PDF)]. The EPA writes: "EPA and the Centers for Disease Control and Prevention (CDC) recognize that certain microorganisms can be ranked with respect to their tolerance to chemical disinfectants. The Spaulding Classification model, used by CDC, tiers microorganisms in accordance with the level of resistance to being killed (inactivation) by typical disinfectant products. With this approach viruses are divided into three viral subgroups (small non-enveloped, large non-enveloped, and enveloped) based on their relative resistance to inactivation. ***According to this hierarchy, if an antimicrobial product can kill a small, non-enveloped virus it should be able to kill any large, non-enveloped virus or any enveloped virus. Similarly, a product that can kill a large, non-enveloped virus should be able to kill any enveloped virus."*** Any tests against any small or large non-enveloped viruses could be used to claim efficacy against enveloped viruses under this guidance. Additionally, any work testing against any other enveloped viruses should also be reviewed as the lipid/protein viral envelope makeup of most enveloped viruses is similar.

Review of Relevant Data

To date, Synexis has performed studies using their DHP technology against two small non-enveloped viruses (Feline Calicivirus and MS-2 Bacteriophage), enveloped influenza viruses (two trials against different strains), and enveloped Infectious Bronchitis Virus (IBV; gammacoronavirus that infects chickens). Each experiment was conducted by an accredited independent laboratory, and the experimental design of each trial was generally the same. Each dataset will be reviewed below. Full study reports are available upon request from Synexis.

Feline Calicivirus (ATCC Strain VR-782). Experiment performed by ATS Labs

Virus inoculum was pipetted onto a glass plate carrier and then allowed to dry. Dried inoculum was then either placed into a biosafety hood that was treated with the Synexis DHP unit or into a non-treated hood. At 0, 1, 2, 6, and 24 hours post placement, 2 mL of media was placed onto the glass carrier and the carrier was scraped to remove any virus still present. Samples were collected in duplicate and averaged for analysis. Recovered virus was tittered in Crandel Reese feline kidney (CRFK) cells and data was reported as tissue culture infectious doses (TCID; Table 1).

Table 1

	Time Post Inoculation (hours)			
	0	2	6	24
Non-Treated	6.6 $\log_{10}$	5.8 $\log_{10}$	5.1 $\log_{10}$	3.4 $\log_{10}$
DHP-Treated	6.6 $\log_{10}$	4.3 $\log_{10}$	2.3 $\log_{10}$	0.6 $\log_{10}$
Percent Reduction (compared to non-treated)	**N/A**	**96.8%**	**99.8%**	**99.8%**

MS2 Bacteriophage (ATCC Strain 15597-B1). Experiment performed by Microchem Laboratory

Phage inoculum was prepared and added to a nebulizer for aerosolization in this study. Nebulizers were turned on and run for 60 minutes to achieve target microbial concentrations in either a control (non-treated) or DHP-treated chamber. At times 0, 1, 2, 3, and 4 hours post inoculation (hpi), an SKC bio-sampler was used to collect air for 40 minutes for each chamber. Samples were then enumerated using standard dilution and plating techniques (24 hours incubation). Since MS2 is a bacteriophage, *E. coli* strain 15597 was used as the permissive host cell system for evaluation of viral load. Data are reported in CFU/m^3 (Table 2).

Table 2

	Time Post Inoculation (hours)				
	0	1	2	3	4
Non-Treated	4.8 $\log_{10}$	3.9 $\log_{10}$	3.3 $\log_{10}$	2.8 $\log_{10}$	2.9 $\log_{10}$
DHP-Treated	4.8 $\log_{10}$	1.2 $\log_{10}$	<1.2 $\log_{10}$*	<1.2 $\log_{10}$*	<1.2 $\log_{10}$*
Percent Reduction (compared to non-treated)	**N/A**	**99.8%**	**99.2%**	**97.3%**	**97.9%**

*Indicates limit of detection for the assay.

H1N1 Influenza Test 1 (Swine Influenza A; ATCC Strain VR-333; A/Swine/Iowa/15/30). Experiment performed by ATS Labs

Virus inoculum was pipetted onto a glass plate carrier and then allowed to dry. Dried inoculum was then either placed into a room (3,672 ft^3) that was treated with the Synexis DHP unit or into a non-treated room. At 0, 0.5, 1, 2, and 3 hours post placement, 2 mL of media was placed onto the glass carrier and the carrier was scraped to remove any virus still present. Samples were collected in duplicate and averaged for analysis. Recovered virus was tittered in Rhesus monkey kidney (RMK) cells and is reported as tissue culture infectious doses (TCID; Table 3).

Table 3

	Time Post Inoculation (hours)				
	0	0.5	1	2	3
Non-Treated	5.6 $\log_{10}$	5.3 $\log_{10}$	5.3 $\log_{10}$	5.3 $\log_{10}$	4.6 $\log_{10}$
DHP-Treated	5.6 $\log_{10}$	5.1 $\log_{10}$	5.0 $\log_{10}$	4.3 $\log_{10}$	3.3 $\log_{10}$
Percent Reduction (compared to non-treated)	**N/A**	**33.9%**	**49.9%**	**90.0%**	**95.0%**

H1N1 Influenza Test 2 (Swine Influenza A; A/PR/8/34). Experiment performed by Antimicrobial Test Laboratories

Virus inoculum was pipetted onto a glass plate carrier and then allowed to dry. Dried inoculum was then either placed into a room (3,672 ft^3) that was treated with the Synexis DHP unit or into a non-treated room for Experiment 1. For Experiment 2, the same procedure was followed, but samples were placed into a DHP-treated hood or a non-treated hood. At 0, 1, and 2 hours post placement, 2 mL of media was placed onto the glass carrier and the carrier was scraped to remove any virus still present. Samples were collected in duplicate and averaged for analysis. Recovered virus was tittered in Madin-Darby Canine kidney (MDCK) cells and data was reported as tissue culture infectious doses (TCID; Tables 4 and 5).

Table 4 (Room)

	Time Post Inoculation (hours)		
	0	1	2
Non-Treated	6.05 $\log_{10}$	4.80 $\log_{10}$	3.80 $\log_{10}$
DHP-Treated	6.05 $\log_{10}$	3.93 $\log_{10}$	3.18 $\log_{10}$
Percent Reduction (compared to non-treated)	**N/A**	**87.0%**	**76.0%**

Table 5 (Hood)

	Time Post Inoculation (hours)		
	0	1	2
Non-Treated	6.05 $\log_{10}$	4.80 $\log_{10}$	3.80 $\log_{10}$
DHP-Treated	6.05 $\log_{10}$	2.18 $\log_{10}$	1.93 $\log_{10}$
Percent Reduction (compared to non-treated)	**N/A**	**99.8%**	**98.6%**

Infectious Bronchitis Virus (GammaCoronavirus; Massachusetts Strain; Mass/M41/M41). Experiment performed by Dr. Brian Jordan, The University of Georgia

Experiment 1. Virus inoculum was pipetted onto a stainless-steel plate carrier and then allowed to dry. Dried inoculum was then either placed into a chamber (48 ft^3) that was treated with the Synexis DHP unit or into a non-treated chamber. At 0, 24, 48, 72, and 96 hours post placement, samples were collected by dipping a cotton swab in the collection media, and then rubbing the swab over the inoculation area on the coupon in at least two different directions. The tip of the swab was then placed back into the collection media (1 mL PBS) and cut off from the swab stem (remaining in PBS media). Samples were then immediately placed into the –80^o C freezer for storage until PCR testing. Viral RNA was extracted from 50 uL of the PBS and real time RT-PCR was conducted according to the manufacturer's recommendations. Data were reported as viral load (in Embryo Infectious Doses 50; EID_{50}) as calculated using the standard curve trendline formula from known titer dilutions (Table 6).

Table 6

	Time Post Inoculation (hours)				
	0	24	48	72	96
Non-Treated	4.80 $\log_{10}$	4.38 $\log_{10}$	4.02 $\log_{10}$	3.91 $\log_{10}$	3.98 $\log_{10}$
DHP-Treated	4.80 $\log_{10}$	3.89 $\log_{10}$	4.04 $\log_{10}$	3.83 $\log_{10}$	3.50 $\log_{10}$
Percent Reduction (compared to non-treated)	**N/A**	**67.7%**	**0%**	**16.8%**	**66.9%**

Experiment 2. Thirty-six (36) glass microscope slides were inoculated with 100ul of the working stock and allowed to dry. Eighteen (18) slides were then moved into an ~150 sq ft room (10x15) being treated with 1 Synexis DHP standalone unit. The remaining glass slides were placed into a laminar flow hood in another lab space with the fan turned off. At placement 3 glass slides were taken out of the room or the flow hood and sampled. Samples were collected by taking a cotton tipped swab, dipping it in the collection media to wet the swab, and then rubbing the swab over the inoculation area on the coupon in at least two different directions. The tip of the swab was then placed back into the collection media (1 ml PBS) and was cut off from the swab stem (remaining in PBS media). Samples were then immediately placed into the -80C freezer for storage until PCR testing. This process was repeated at 12, 24, 48, 72, and 96 hours post inoculation. Data were reported as viral load (in Embryo Infectious Doses 50; EID_{50}) as calculated using the standard curve trendline formula from known titer dilutions (Table 7).

Table 7.

	Time Post Inoculation (hours)					
	0	12	24	48	72	96
Non-Treated	3.19 log_{10}	3.08 log_{10}	3.17 log_{10}	3.24 log_{10}	3.05 log_{10}	2.81 log_{10}
DHP-Treated	3.19 log_{10}	2.25 log_{10}	1.05 log_{10}	1.35 log_{10}	1.34 log_{10}	1.39 log_{10}
Percent Reduction (compared to non-treated)	**N/A**	**85%**	**99.2%**	**98.9%**	**98%**	**96.1%**

Discussion

For the two non-enveloped viruses, Feline Calicivirus and MS2 Macrophage, the Synexis DHP system easily achieved at least a 99% reduction in viral load over natural die off (comparing to a non-treated control to determine natural die off) within a few hours (1-6 maximum). This is very promising data since small non-enveloped viruses are the hardest to kill with disinfectants. Furthermore, this data was compiled through aerosolization as the route of inoculation, the main route of transmission for respiratory viruses, as well as on hard non-porous surfaces, which are known to be reservoirs for pathogens including the novel SARS-CoV-2 virus. With this data, the Synexis DHP system would be qualified as effective against SARS-CoV-2.

For the trials with enveloped viruses, including a member of the Coronavirus family, the data show that a minimum reduction in viral load of 87%, and up to 99%, was achieved for Influenza H1N1 in a short time (1-3 hours). The viral load reduction over natural die off for IBV, of the Coronavirus family, was 67% at 24 hours in Experiment 1, which is also significant. After 24 hours however, the reductions are lost and there is no real difference between the treated and non-treated groups until 96 hours when the same 67% reduction is again seen. This experiment was influenced by poor environmental conditions for the efficacy of DHP. A small plexiglass chamber was used, which we have realized is not enough air space to effectively create and maintain DHP levels. This was seen when measuring DHP levels of 0ppb with the Interscan

hydrogen peroxide detector prior to beginning the experiment. At this point, we also realized that there was not enough fresh air flow into the chamber to create DHP, so more air was allowed into the chamber by creating a gap around the intersection of the plexiglass and the floor to allow more air in. When this was done, the Interscan began reading levels of 10ppb for DHP so the experiment was carried forward. By 24 hours however, the Interscan was gain reading 0ppb and did so for the remainder of the experiment. Because of this it can stated that no timepoints past 24 hours are a valid comparison because no DHP could be detected. Contrast this to Experiment 2 where robust reductions in viral load were seen within 12 hours, but definitely within 24 hours. For this experiment, a much larger space was used with natural air inflow. This allowed the DHP unit to work more efficiently and produce DHP. Interscan readings for Experiment 2 were 9-11ppb in the first 24 hours and then 11-14ppb for the remainder of the experiment. With appropriate levels of DHP production, a 99% reduction in viral load was achieved within 24 hours.

It is also important to note that there were differences in the IBV study when compared to the other experiments. The previous studies utilized *in-vitro* titration assays to determine viral load. For the IBV study, real-time reverse transcriptase PCR (qRT-PCR) was used to estimate viral load. qRT-PCR detects the presence of viral genetic material, which means it may also detect intact genetic material of dead viruses. The fact that there was a reduction in qRT-PCR values used to calculate viral load indicates that not only were viruses dead, but that they had been destroyed rapidly enough to allow time for genetic material degradation as well. It may be hypothesized that an actual *in-vitro* titration of the samples collected would show that there is much less live virus present than the qRT-PCR indicated. Unfortunately, the *in-vitro* titration could not be performed at this time due to the cessation of all research activity and closing of the university where the test was performed due to the SARS-CoV-2 pandemic.

Concluding Statement

Herein, 6 separate trials have been detailed proving the ability of the Synexis DHP system to reduce viral load of small non-enveloped and enveloped viruses through aerosol inoculations and on hard non-porous surfaces. This data shows that DHP may be used to combat the novel SARS-CoV-2 pandemic currently devastating the world.

References

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