BIOGLOVE
MICROBIAL DEFENSE SYSTEMS

Compared to Alcohol Sanitizers

General Info / Facts:

- Alcohol sanitizers are ineffective when applied to hands that are not completely clean. Alcohol cannot penetrate the film of grease, dirt, or oil on the epidermis thus making them ineffective
- Alcohol sanitizers can quickly reduce the number of germs on your hands, but since alcohol evaporates within 30 seconds, it leaves hands vulnerable and unprotected until the next application
- Frequent use of alcohol gel sanitizers leaves a bio film on skin surfaces that actually traps dirt and fosters the growth of germs and bacteria
- Alcohol hand sanitizers are only effective if they contain more than 60% alcohol
- Alcohol hand sanitizers contribute to drying and chapping of hands
- Traditional hand sanitizers are highly flammable and can cause alcohol poisoning if ingested

BioGlove PBS Details:

MRSA: Methicillin Resistant Staphylococcus Aureus

- MRSA is a type of Staph bacteria that can cause very serious bacterial infections
- It's a superbug, resistant to most antibiotics such as Methicillin, Oxacillin, Penicillin, and Amoxicillin
- Constantly increasing resistance to antibiotics
- Spread by contact
- Responsible for an estimated 20,000 deaths each year (cdc.gov)

BioGlove PBS is effective against MRSA

Clostridium difficile (C. Difficile)

- Bacterium that can cause symptoms ranging from diarrhea to life-threatening inflammation of the colon
- 9,000 cases of C.diff are hospital-acquired (cdc.gov)
- Responsible for an estimated 28,000 deaths annually (cdc.gov)
- Bacterium can exist as dormant spores and survive months on hard surfaces
- Alcohol hand sanitizers don't kill C. diff and are ineffective against the spores
- Soap and water cannot kill C. diff in spore form

BioGlove PBS is effective against C. diff

Norovirus “the stomach bug”

- Highly contagious for up to two weeks; notorious for outbreaks on cruise ships, schools, nursing homes
- Causes acute gastroenteritis; the most common symptoms are diarrhea, vomiting, and stomach pain
- Leading cause of infectious outbreaks in hospitals in the US (American Journal of Infection Control)
- Bacterium can exist as dormant spores and survive months on hard surfaces
- Affects more than 20 million Americans every year, causing an estimated 800 deaths (cdc.gov)
- There is no vaccine and no treatment for norovirus

BioGlove PBS is effective against norovirus

H1N1: Influenza or the flu virus

- Highly contagious
- Spreads mainly person-to-person through coughing or sneezing of infected people
- Flu vaccines can cause flu-like symptoms, allergies, or side effects, and do not protect against all strains of influenza
- Over 60 million cases annually (cdc.gov)
- Over 12 million deaths from April 2009 - April 2010 (cdc.gov)

BioGlove PBS is effective against H1N1

**** Important to note: 80% of infectious diseases are transmitted by touch (WebMD)****

BioGlove®	✓	Alcohol Sanitizer	Purell®
12+ Hr Protection with One Application	**Yes!**	No	Protects only for 30 seconds until alcohol evaporates
Complete Non-Alcohol Formulation	**Yes!**	No	Contains 60+% Alcohol
Kills Bacteria, Viruses, Fungi on Contact	**Yes!**	No	Yes kills some, Not all
Gently Moisturizes Sensitive Skin	**Yes!**	No	Alcohol causes drying, chaffing, and cracking
Prevents Pathogen Mutation (Superbugs)	**Yes!**	No	Encourages superbugs in some circumstances
Non-Toxic & Non-Flammable	**Yes!**	No	Highly Toxic and Highly Flammable
Clean, Refreshing Scent	**Yes!**	No	Unpleasant Alcohol Odor
All-Day Germ Protection for Pennies	**Yes!**	No	Comparable Protection is VERY Expensive
Antimicrobial protection even when applied to soiled, dirty, greasy hands	**Yes!**	No	Alcohol cannot penetrate the dirt or oily layer and is ineffective

ISU Study

Antibacterial Efficacy of a Novel Antimicrobial Skin Cleanser against Food borne Enteric Pathogens on a Model Skin Surface

Part 1: Antibacterial Efficacy of a Novel Antimicrobial Skin Cleanser against Food-borne

Materials and Methods

Strains and media. All aseptic techniques and handling of microbial cultures were performed in a Nuaire Biological Safety Cabinet (Model #NU-425-400). Five strains each of Escherichia coli O157:H7 and Listeria monocytogenes were used in this study. Each strain was isolated either from infected processed food or human infection (Table 1). E. coli O157:H7 was grown at 35oC on tryptic soy broth + 0.6% yeast extract (TSB/YE) (Difco Laboratory, Detroit, MI); L. monocytogenes was grown at 30oC on brain heart infusion (BHI) (Difco Laboratory). E. coli O157:H7 and L. monocytogenes cell counts were made on sorbitol-MacConkey agar (SMA) (Difco Laboratory) and modified Oxford medium (MOX) (Difco Laboratory), respectively. For all experiments, a 5-strain mixture ("cocktail') of the specific test organism was used. Five ml of an overnight culture of each strain were aseptically combined in a sterile 50-ml polypropylene centrifuge tube. The cells were harvested by centrifugation (10,000 x g at 4oC for 10 min) in a Sorvall Super T 21 centrifuge with an SL-50T rotor. The supernatant was discarded and the pelleted cells were resuspended and washed in 25 ml of either sterile 0.85% saline or sterile distilled water. The washed cells were harvested as previously mentioned and the supernatant discarded. The cells were resuspended in 25 ml of either sterile 0.85% saline or sterile distilled water and used as inocula for sanitizer evaluations.

Introduction

Sanitation of hands is a major intervention for preventing the transmission of pathogens to food, food contact surfaces, or other areas that must be kept sanitary in food processing, food service, clinical or veterinary environments. Of late, there has been **a growing trend in the demand for sanitizers that may be applied to hands without the need for subsequent rinsing.** Many hand sanitizers contain ethanol as the active antimicrobial, along with moisturizers and emollients to keep the skin from becoming dry. While ethanol will exert an antimicrobial effect, its rapid evaporative loss from the skin reduces the effectiveness of many hand sanitizers in providing residual antimicrobial protection. Recently, hand sanitizers (Pure bioguard) containing silylated quaternary ammonium compounds (QACs) have been developed. QACs are well known to have antimicrobial activity that is manifested by disruption of microbial cell membranes, resulting in leakage of intracellular metabolites and cell death (1). Silylated QACs have the ability to react with and chemically bond to inanimate surfaces such as cellulose fabrics ([2, 3, 4]), glass ([3, 6]), zeolite ([1]), etc., with retention of antimicrobial activity. They can also bond to skin (5). When applied to the hands, quaternary ammonium organosilanes **bind to the skin via covalent-, electrostatic-, or hydrogen bonds, forming an antimicrobial layer to inactivate existing skin pathogens as well as pathogens that contaminate the skin long after application of the sanitizer.** In this communication, we report the effectiveness of these sanitizers for killing human enteric pathogens on inanimate surfaces (polystyrene Bioscreen plates) with retention of antimicrobial activity for extended periods in a rapid microliter plate assay.

Table 1. Pathogens used for the evaluation of sanitizers1

Organism	Isolate	Source
L. monocytogenes	FSL J1-177	Human sporadic case (1997)
“	FSL C1-056	Human sporadic case (1998)
“	FSL N3-013	Food isolate from listeriosis epidemic (UK, 1988-2000)
“	FSL R2-499	Human isolate from sliced turkey outbreak (USA, 2000)
“	FSL N1-227	Food isolate (US, 1998-1999)
E. coli O157:H7	FRIK 125	Dr. C. Kaspar, Univ of Wisconsin, Madison
“	ATCC 43890	Human feces isolate, ATCC2
“	ATCC 43895	Ground beef isolate, ATCC
“	C-467	Dr. C. Kaspar, Univ of Wisconsin, Madison
“	93-062	Culture collection, Microbial Food Safety Lab. FSHN

1. Culture collection, Microbial Food Safety Lab. FSHN
2. American Type Culture Collection

Sanitizers. The sanitizers BioGlove PBS and BioGlove PB64 contain silylated quaternary ammonium compounds as their active ingredients. Also included was a Silicate Test Solution containing an inert organosilane compound that served as a control. For comparison, 3 commercially-available sanitizers were also provided: 1) 2 in 1 HandClens; 2) Dial Hand Sanitizer; 3) Purell Instant Hand Sanitizer. Other solutions used in the trials were sterile distilled water and 62% (w/w) ethanol. The sanitizers tested and their ingredients are listed in Table 2.

Table 2. Sanitizers evaluated

Sanitizer	Active Ingredient	% Ethanol
Silicate Test Solution	Methyl-Trihydroxy-Silane	18
BioGlove PBS	0.5% 3-(trihydroxysilyl) propyl-dimethyl-octadecyl Ammo. Cl	18
BioGlove PB64	0.5% 3-(trihydroxysilyl) propyl-dimethyl-octadecyl Ammo. Cl	62
2 in 1 HandClens	3% Benzalkonium Chloride	0
Dial Hand Sanitizer	Ethanol	62
Purell Hand Sanitizer	Ethanol	62

1. Contains 3-(trihydroxysilyl) propyl-dimethyl-octadecyl ammonium chloride

Rapid Bioscreen assay for antimicrobial activity.

The retention of antimicrobial activity of sanitizers on inanimate surfaces was tested using a Microbiology Reader Bioscreen C (Oy Growth Curves AB Ltd., Helsinki, Finland). This instrument continuously monitors absorbency changes of bacterial cultures at specific wavelengths, temperature and shaking speeds in polystyrene plates containing 100 micro liter wells. Evaluations were performed as follows: Sixty µL of each sanitizer were added to separate wells of sterile Bioscreen plates. Plates were dried in a 36-38oC incubator overnight, after which 10 µL of a 1:100 dilution of washed cells of a 5- strain mixture of E. coli O157:H7 or L. monoctyogenes were added to the Bioscreen wells, followed by 250 µL of sterile media. The plates were inserted in the Bioscreen and incubated at 30oC for periods of 2-3 days. The plates were shaken at low speed for 10 sec before hourly absorbency readings (A600) were made. All sanitizers were evaluated in replicates of 5. Growth curves were obtained from the Bioscreen data using Excel.

Results and Discussion Part 1. Antimicrobial effect of sanitizers to pathogens on inanimate surfaces. Figure 1 compares the growth of E. coli O157:H7 and L. monocytogenes in Bioscreen plate wells with and without exposure to sanitizers. In this experiment, sanitizers were added to the Bioscreen plate wells, and the plate dried at 48-49°C. After 19 hours, inoculate were added to the plate wells and the plate incubated at 37°C and ~8 hr to dry the inoculate, followed by adding 250 uL of sterile media to each well. The plate was then incubated in the Bioscreen at 30°C for 62 hours.

Figure 1 compares the growth of E. coli O157:H7 and L. monocytogenes in Bioscreen plate wells with and without exposure to sanitizers. For both organisms, normal growth was observed in the absence of sanitizers and with cells exposed to distilled water. Cells exposed to wells pretreated with 62% ethanol, Dial-, and Purell sanitizers also grew normally. The latter contain ethanol as their active ingredient. Evaporative loss of ethanol from these wells eliminated the anti-microbial efficacy of these treatments.

Normal growth of both organisms was observed in wells treated with the Silicate Solution. This is identical to BioGlove PBS, but contains an inert organometallic derivative. Neither organism was able to grow following exposure to BioGlove PBS PB, PB64 and 2 in 1 HandClens. The active ingredients in these products (Table 2) are not volatile and, after the drying step, remain to coat the wells and prevent growth of the microbes following inoculation. At the end of the incubation period, 50 µL aliquots from wells treated with these sanitizers were aseptically transferred to tubes of sterile broth and incubated at a suitable temperature. After 2 days, no growth was observed, demonstrating that absence of growth in the Bioscreen plate was not due to inhibition, but to death of the cells exposed to the sanitizers coating the wells. The results also demonstrate that the active ingredient in PB and PB64 (3-(trihydroxysilyl)-propyl-dimethyloctadecyl ammonium chloride) is active against Gram positive and Gram negative bacteria. This is in agreement with the findings of others (3, 4).

Profiles obtained with 2 in 1 HandClens show an initial high absorbency, followed by a decrease over the incubation period. This is especially apparent in wells containing L. monotonousness in BHI medium. This was tested and found to be due to some interaction between one or more of the sanitizer components with the medium, and not to an effect on the inocula. To preclude the possibility that drying the Bioscreen plate at ~49°C prior to inoculation caused thermal decomposition of the sanitizers, the experiment described in Figure 1 was repeated, with the following modifications: (a) the sanitizers were dried in the Bioscreen plate for 23 hrs at ~37°C; (b) the inocula were not taken to dryness prior to addition of media, but instead were incubated in the Bioscreen plate for only 1 hr at 37°C prior to addition of 250 µL of growth media. The results are shown in Figure 2. The results from this trial were essentially identical to those obtained from the first experiment, confirming the observations from the latter. Thus, exposure of the sanitizers to ~50°C does not cause thermal decomposition. Moreover, the active ingredients in Pure bioguard 64 and 2 in 1 HandClens were biocidal even though the inocula did not go to dryness. This shows that failure of the pathogens to grow in wells treated with these sanitizers was not due to desiccation-induced death of the cells.

Summary of Results

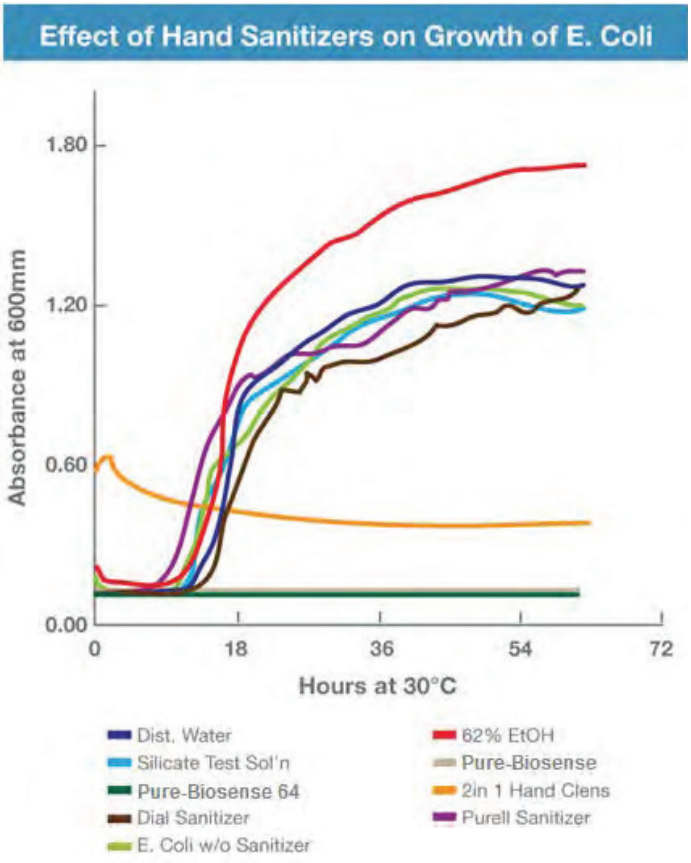
E. coli O157: H7 and L. monocytogenes grew normally (growth similar to control) in broth medium added to polystyrene wells that were pre-treated with 62% ethanol, Dial or Purell sanitizers. The observed lack of growth inhibition is likely due to the evaporative loss of ethanol from these treatment solutions.

Normal growth of both pathogens also occurred in wells pre-treated with silicate solution.

No growth of either pathogen was observed in wells that were pre-treated with PB, PB64, or 2 in 1 HandClens. The active antimicrobial agents in these sanitizers are not volatile and persisted in the wells (following the drying step) to inhibit growth of the pathogens.

PB, PB64 and 2 in 1 HandClens exerted a bactericidal effect on both pathogens. BioGlove PBS and BioGlove PBS 64 both contain 3-(trihydroxysilyl)-propyl-dimethyloctadecyl ammonium chloride as their active ingredient.

Data from Bioscreen evaluations show that the BioGlove PBS 64 formulations are active against Gram positive and Gram negative organisms. This is in agreement with findings of others (3,4).



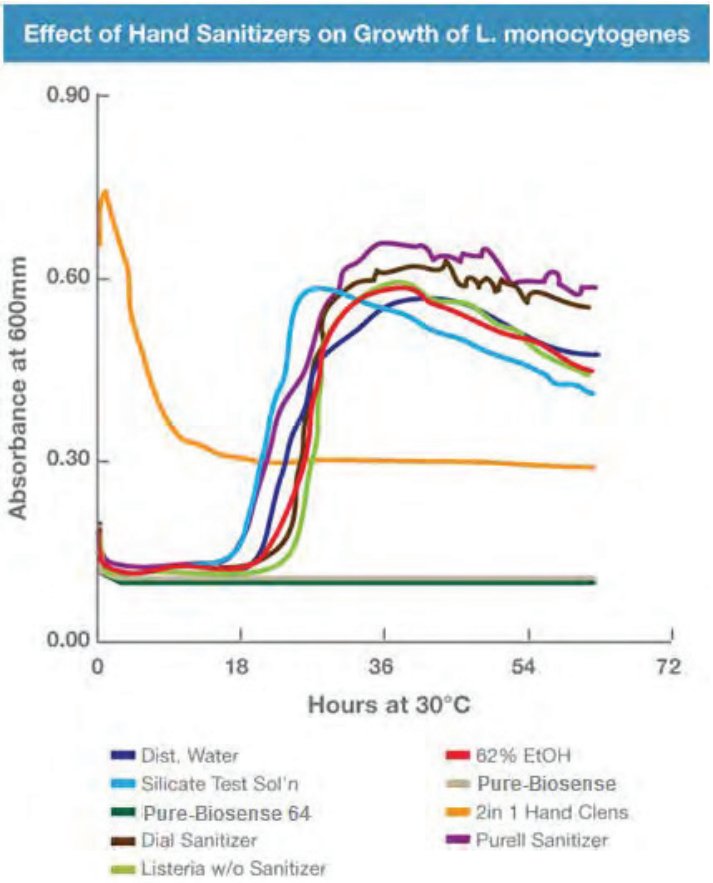


Figure 1

** Pure-BioSense is BioGlove PBS Hand Santizer, Pure-BioSense 64 is BioGlove PB64 Surface Sanitizer*

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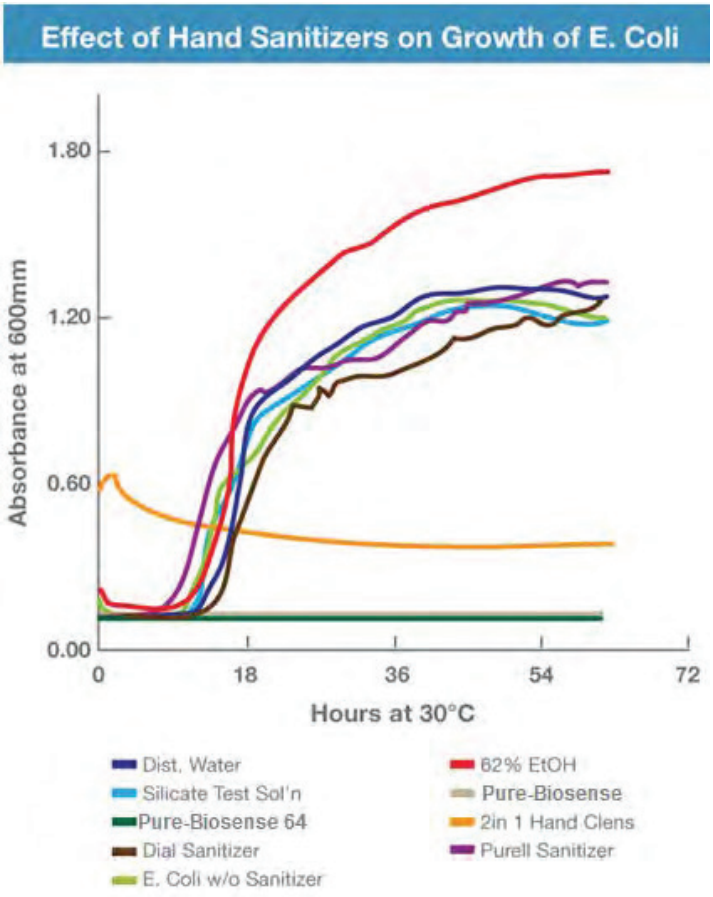
Fig. 1. **BioGlove** PBS and BioGlove PB64 Growth of E. coli O157:H7 and L. monocytogenes in Bioscreen plates with and without sanitizer treatment. Sanitizers were added to wells and the plate dried overnight at ~49°C. Five-strain "cocktails" of E. coli O157:H7 and L. monocytogenes were used as inocula. Cells were harvested and washed as described in Methods, resuspended in sterile water to one-half their original volume, then diluted 1:100 with sterile water. Inocula (10 µL) were added to wells, then dried for 8 hrs at 37-38°C. Next, 250 µL of sterile media were added to all wells. The plate was incubated in the Bioscreen at 30°C for 62 hrs (n=5). "Green Flat line"



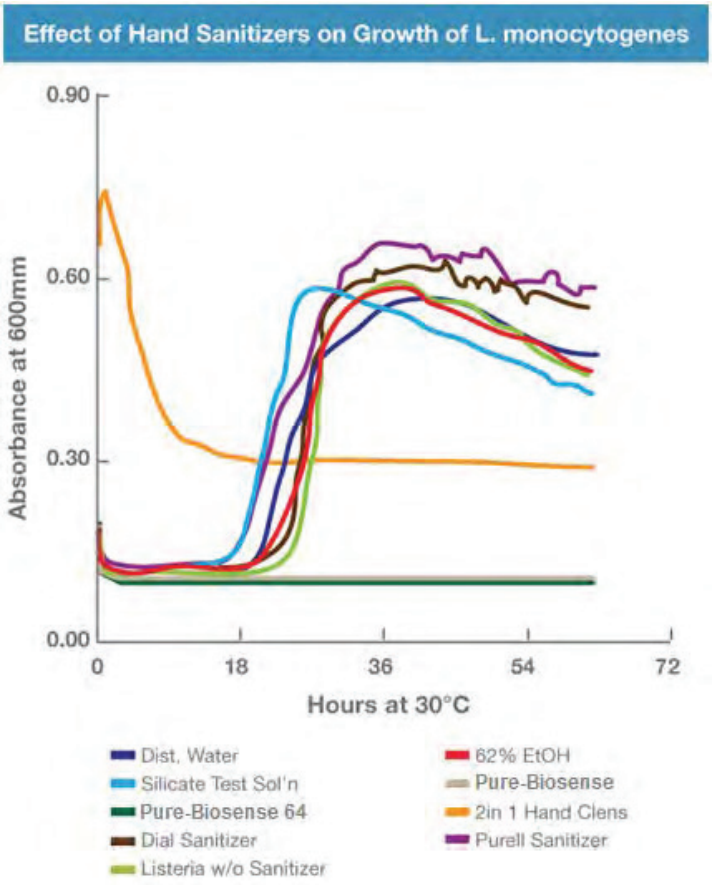


Figure 2

BIOGLOVE®

Fig. 2. BioBlove PBS and BioGlove PB64 Growth of E. coli O157:H7 and L. monocytogenes in Bioscreen plates with and without sanitizer treatment. Sanitizers were added to wells and the plate dried overnight at ~37°C. Five-strain "cocktails" of E. coli O157:H7 and L. monocytogenes were used as inocula. Cells were harvested and washed as described in Methods, resuspended in sterile water to one-half their original volume, then diluted 1:100 with sterile water. Inocula (10 µL) were added to wells, after which the plate was incubated at 37°C for 60 min. Next, 250 µL of sterile media were added to all wells. The plate was incubated in the Bioscreen at 30°C for 52 hrs (n=5). "Green Flat Line"

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MICROBIAL DEFENSE SYSTEMS

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Corona virus	
Virus	Corona virus
Structure	Enveloped
Family	Coronaviridae
Host(s)	Humans
Disease(s) Caused	Mild to severe respiratory illness
Symptoms	Cough, runny/stuffy nose, fever, sore throat, headache, diarrhea
Potential Complications	Pneumonia, death
Potential Complications	Close person-to-person contact, contaminated fomites
Sites of Community Outbreaks	Schools, hospitals, daycare centers

Importance of Corona viruses

Two strains of human corona virus, 229E and OC43, are known to cause approximately 25% of colds that exhibit symptoms similar to those caused by the rhinoviruses (e.g. runny nose, sneezing and cough). However, recent zoonotic strains of corona virus characterized by species-jumping from animals to humans have gained notoriety and become of particular concern over the past decade. The SARS-CoV (Severe Acute Respiratory Syndrome corona virus) outbreak of 2002-2003 originated in bats and spread indirectly to humans via intermediate animals (e.g. civet cats). From the earliest reported cases in southern China, the virus eventually spread to 28 countries over the course of eight months; thousands are believed to have been infected and 774 deaths were reported **(1). The MERS-CoV (Middle East Respiratory Syndrome Corona virus) outbreak originating in Saudi Arabia in April of 2012 made headlines due to its high mortality rate of 45% and rapid spread to 9 countries (6); clusters of cases have continued to be reported in the Middle East through the end of 2020.**

The Importance of Disinfection: Survival of Corona viruses on Surfaces and Transmission Potential via Fomites

The zoonotic SARS corona virus strain demonstrated both respiratory and intestinal replication routes for human hosts. A retrospective study of 138 patients infected with SARS-CoV found that almost 40% of patients developed diarrhea, and that SARS-CoV genomic material was detectable in the stool of patients for more than 10 weeks after onset of the initial illness (4). The release of infectious SARS viruses not only into the air, but also into the water supply, further amplified the need for an effective halt to potential environmental transmission. Relative to strain 229E, SARSCoV maintains infectivity longer in suspension and when dried onto surfaces, and is also more thermally resistant (30 minutes at 56°C and 60°C) in the presence of 20% fetal calf serum (8). Although SARS-CoV appears to be more environmentally resistant relative to the respirator corona viruses, its enveloped structure is still vulnerable to a wide range of disinfectants. Suspension evaluations of propanol (100 % and 70%) and ethanol (78%) demonstrated reduction of SARS-CoV to levels below detection in 30 seconds; 60 seconds were required for wine vinegar and 120 seconds for formaldehyde (0.7% and 1%) and 0.5% glutardialdehyde (8). Povidone-iodine (PVP-I) products, **quaternary ammonium compounds** “contained in BioGlove PBS”, free chlorine, and catalytic oxidation via Ag/**Al2O3 and Cu/Al2O3 active surfaces have also been proven to completely inactivate SARS-CoV (2, 3, 9, 12). Therefore, environmental transmission of corona viruses via fomites and liquids can be minimized given the proper** implementation of disinfection protocols.

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MICROBIAL DEFENSE SYSTEMS

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National Institutes of Health

COVID-19 is an emerging, rapidly evolving situation.

Get the latest public health information from CDC: https://www.coronavirus.gov
Get the latest research information from NIH: https://www.nih.gov/coronavirus

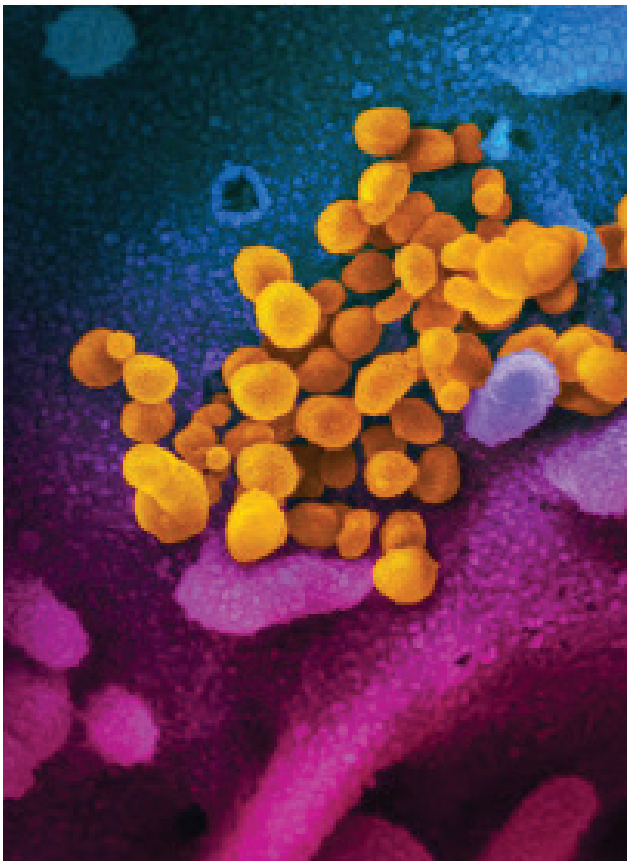
NEWS RELEASES

Tuesday, March 17, 2020

New corona virus stable for hours on surfaces

SARS-CoV-2 stability similar to original SARS virus.

What



The virus that causes corona virus disease 2019 (COVID-19) is stable for several hours to days in aerosols and on surfaces, according to a new study from National Institutes of Health, CDC, UCLA and Princeton University scientists in The New England Journal of Medicine. The scientists found that severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was detectable in aerosols for up to three hours, up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel. The results provide key information about the stability of SARS-CoV-2, which causes COVID-19 disease, and suggests that people may acquire the virus through the air and after touching contaminated objects. The study information was widely shared during the past two weeks after the researchers placed the contents on a preprint server to quickly share their data with colleagues. The NIH scientists, from the National Institute of Allergy and Infectious Diseases' Montana facility at Rocky Mountain Laboratories, compared how the environment affects SARS-CoV-2 and SARS-CoV-1, which causes SARS. SARS-CoV-1, like its successor now circulating across the globe, emerged from China and infected more than 8,000 people in 2002 and 2003. SARS-CoV-1 was eradicated by intensive contact tracing and case isolation measures and no cases have been detected since 2004. SARS-CoV-1 is the human corona virus most closely related to SARS-CoV-2. In the stability study the two viruses behaved similarly, which unfortunately fails to explain why COVID-19 has become a much larger outbreak. The NIH study attempted to mimic virus being deposited from an infected person onto everyday surfaces in a household or hospital setting, such as through coughing or touching objects. The scientists then investigated how long the virus remained infectious on these surfaces.

The scientists highlighted additional observations from their study:

- If the viability of the two corona viruses is similar, why is SARS-CoV-2 resulting in more cases? Emerging evidence suggests that people infected with SARS-CoV-2 might be spreading virus without recognizing, or prior to recognizing, symptoms. This would make disease control measures that were effective against SARS-CoV-1 less effective against its successor.

- In contrast to SARS-CoV-1, most secondary cases of virus transmission of SARS-CoV-2 appear to be occurring in community settings rather than healthcare settings. However, healthcare settings are also vulnerable to the introduction and spread of SARS-CoV-2, and the stability of SARS-CoV-2 in aerosols and on surfaces likely contributes to transmission of the virus in healthcare settings.

The findings affirm the guidance from public health professionals to use precautions similar to those for influenza and other respiratory viruses to prevent the spread of SARS-CoV-2:

- Avoid close contact with people who are sick.
- Avoid touching your eyes, nose, and mouth.
- Stay home when you are sick.
- Cover your cough or sneeze with a tissue, then throw the tissue in the trash.
- Clean and disinfect frequently touched objects and surfaces using a regular household cleaning spray or wipe.

Article
N van Doremalen, et al . Aerosol and surface stability of HCoV-19 (SARS-CoV-2) compared to SARS-CoV-1. The New England Journal of Medicine . DOI:
10.1056/NEJMc2004973 (2020).

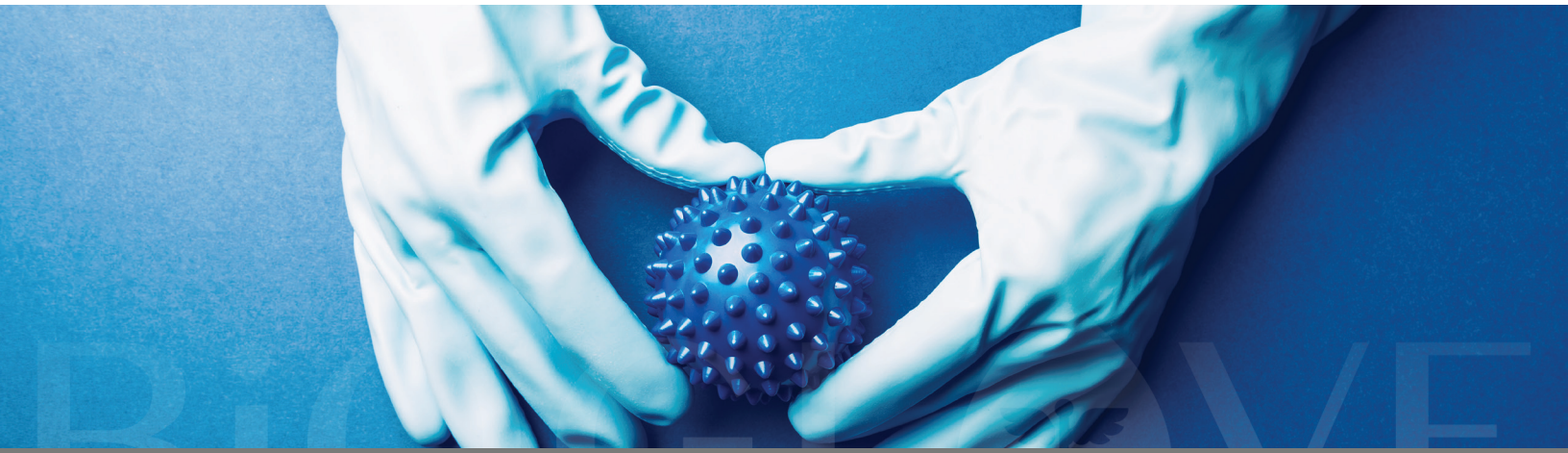
Who
NIAID Director Anthony S. Fauci, M.D., and Vincent Munster, Ph.D., a principal investigator in NIAID's Laboratory of Virology, are available to comment on this study.

This media availability describes a basic research finding. Basic research increases our understanding of human behavior and biology, which is foundational to advancing new and better ways to prevent, diagnose, and treat disease. Science is an unpredictable and incremental process— each research advance builds on past discoveries, often in unexpected ways. Most clinical advances would not be possible without the knowledge of fundamental basic research.

NIAID conducts and supports research — at NIH, throughout the United States, and worldwide — to study the causes of infectious and immunemediated diseases, and to develop better means of preventing, diagnosing and treating these illnesses. News releases, fact sheets and other NIAIDrelated materials are available on the NIAID website.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

NIH...Turning Discovery Into Health precautions



EN TESTING RESULTS FOR (PBS) PUREBIOSENSE
THE FOLLOWING RESULTS ARE A COMPILATION OF RECENT TESTING COMPLETED ON PBS FOLLOWING EUROPEAN (EN) TESTING METHODS AND PROTOCOLS.

Test Date: 08/31/2009 EN1276
Test Material: BioGlove PBS
Test: EN1276, Suspension test evaluation of bactericidal activity for a chemical disinfectant.
Test Results: PASS
Test Organism:

	P. aeroginosa	E. coli	Staphylococcus. aureus	Enterococcus hirae
Log reduction:	6.71	6.42	5.94	5.85

Conclusion: According to EN 1276, PBS when used neat as received possesses satisfactory bactericidal activity in 5 minutes at 20 degrees C under dirty conditions (3.0g/l bovine albumin) for the reference organism to be an effective chemical disinfectant.

Test Date: 08/31/2010 EN1650
Test Material: BioGlove PBS
Test: EN 1650, Quantitative suspension Test for evaluation of fungicidal activity of chemical disinfectants.
Test Results: PASS

Test Organism:	Candida albicans	Aspergillius niger
Log reduction	6.48	4.09

Conclusion: According to EN 1650, PBS when used neat, possesses satisfactory fungicidal activity in 15 minutes at 20 degrees C under dirty conditions (3.0g/l bovine albumin) for the referenced organisms.

Test Date: 06/06/2012 EN13624
Test Material: BioGlove PBS
Test: EN13624, chemical disinfectants and antiseptics. A quantitative suspension test for the fungicidal and yeasticidal activity of chemical disinfectants for instruments used in the medical area.
Test Results: PASS

Test Organism:	Candida albicans
Log reduction:	>4.21

Conclusion: PBS when used neat passes the requirements of EN 13624 for yeasticidal activity in 60 minutes at 20 degrees C under conditions (3.0 g/l bovine albumin) against the referenced organism.

Test Date: 06/06/2012 EN13727
Test Material: BioGlove PBS
Test: EN13727, chemical disinfectants and antiseptics- quantitative suspension test for the evaluation of bactericidal activity of chemical disinfection for instruments used in the medical area.
Test Results: PASS

Test organism:	P. aeroginosa	S. aureus	E. hirae
Log reduction:	5.05	5.09	5.05

Conclusion: PBS when used neat, passes the requirements of EN13727 for bactericidal activity in 5 minutes under dirty conditions (3.0 g/l bovine albumin) against the referenced organisms.

Test Date: 06/06/2012 EN13704
Test Material: BioGlove PBS
Test: EN13704, Chemical disinfectants. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in human medicine, veterinary field and food, industrial, domestic and institutional areas.
Test Results: PASS

Test organism:	Clostridium dificile (spore)
Log reduction:	>3.35

Conclusion: PBS when used neat, passes the requirements of EN13704 for sporicidal activity in 60 minutes at 20 degrees C under clean conditions against the reference organism.

Test Date: 06/07/2012 EN1500 (15 Subjects)
Test Material: BioGlove PBS
Test: EN1500, Chemical disinfectants and antiseptics- Hygienic hand-rub- Test Method and requirements. For the test product to pass criteria of EN1500 the mean log reduction factor obtained must not be significantly smaller that that obtained for the 2-propanol (IPA). Principle of the Test: The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after using the hygienic hand-rub. The ratio of the two resulting values is called the reduction factor and represents a measure of the antimicrobial hygienic hand-rub product tested. In order to achieve the necessary precision a large number of subjects has to be used because of the possible variation of bacterial flora found on human skin. Pre-values Determination: Each of the 15 subjects washed their hands in soft soap for 1 minute and dried them on a paper towel. The hands were then contaminated by immersion with high concentration of E. coli (106 CFU/ML) and allowed to air for 3 minutes. Each of the subjects rubbed their fingertips and thumb on the base of a petri dish to assess the release of test organisms. Hand-rub Procedure: Each subject poured 3 ml 0f 2-propanol onto cupped hands and rubbed vigorously using hand-over-hand procedure for 30 seconds. Procedure repeated with additional 3 ml 2-propanol. Total rubbing time 60 seconds. Hands were rinsed under running tap water. The foregoing procedure was repeated exactly using the PBS test product in place of 2-propanol. Post Values: Immediately after rinsing, the 15 subjects rubbed their fingertips on the base of a petri dish containing 10 ml MRD. Plates were incubated and examined fro growth of the test organism.

Test Results: PASS (BioGlove PBS is more effective at bacterial reduction than IPA).

Test organism:	Escherichia coli (IPA)	Escherichia coli (PBS)
Log reduction (Average):	5.64	5.69
Standard deviation:	0.49	0.52

Conclusion: BioGlove PBS passes the requirements of EN1500 for hygienic hand sanitizer when tested under the procedure described.

Test Date: 06/11/2012 EN12791 (20 Subjects- 24 hr. Adaptation)
Test material: BioGlove PBS
Test: Adaptation of EN12791, chemical disinfectants and antiseptics- surgical hand disinfection- Test method and requirements to show immediate effect and continuing effect after 24 hours.
Principle of Test: The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after using the hygienic hand-rub. In order to achieve the necessary precision, a large number of subjects have to be used because of the variation of flora on human skin. In this study a total of 20 healthy adults were chosen, each one carrying out the test procedure in precisely the same way as the others. To compensate for extraneous influences the test sample reduction factor is compared with the reduction factor obtained with a parallel reference hand-rub procedure which is performed with the same subjects on the same day and under comparable environmental conditions.

Pre-Values Determination: Each of the 20 subjects washed their hands for 1 minute in soft soap to remove natural commensal organisms and dry them thoroughly on a paper towel. The fingertips including thumbs were then rubbed for 1 minute on the base of a petri dish containing 10 ml Tryptone Soy Agar (TSB) without neutralizer to assess the release of skin bacteria before treatment of hands (pre-values). A separate dish was used for each hand. The plates were incubated for enumeration. Immediately after sampling the reference disinfection (IPA) or the procedure under test (PBS) is performed.

Reference Surgical Hand Disinfection Procedure (2-propanol): Each of 10 subjects poured 3 ml of 2-propanol (IPA) into cupped hands and using the standard hand-over-hand hand-rub procedure to apply the disinfectant. This procedure was repeated with an additional 3 ml of the IPA for a total of 60 seconds. After 60 seconds, the hands were rinsed under running tap water for 5 seconds, excess water is shaken off.

Surgical Hand Disinfection Procedure with PBS: The above procedure was repeated exactly with a second group of 10 subjects using PBS in place of 2-propanol.

Post Values, Immediate: Immediately after rinsing, the 20 subjects were asked to rub the fingertips on the base of a petri dish containing 10 ml TSB with neutralizer for 1 minute using a separate dish for each hand. Then 1 ml of the TSB sample fluids was placed in a petri dish and covered with 15 ml of TSA, mixed, allowed to set and incubated for enumeration of the test organism. This afforded ten results for the reference product (60% IPA) and ten results for the PBS test product.
Each of the two groups were then sent about their normal business for 24 hours and asked to wash their hands only the minimum number of times for personal hygiene reasons during that period.
Post Value, 24-hour: After a 24 hour period each of the two groups was retested as above. This is the post value 24-hour count.

Test Results: PASS (BioGlove PBS exceeds IPA as a hygienic hand sanitizer).
Test Organism: Normal Skin Flora

Test Material Time:	Immediate	24 hours
Log Reduction: 2- propanol (60%)	2.56	0.03
Log Reduction: PBS	2.78	1.12

The above values are averaged for the 20 subjects with a standard deviation of 0.16 and O.22.
Conclusion: The PBS passes the requirements of EN12791 for surgical hand disinfectants for it immediate effect when tested under the procedure described above.

The PBS continues to demonstrate residual antimicrobial on the hands after 24 hours of normal activity while the reference product (IPA) shows little or no activity after this time.

Test Date: 09/08/2009 EN1500 (12 Subjects)
Test material: BioGlove PBS
Test: EN1500, Chemical disinfectants and antiseptics- hygienic hand-rub, test method and requirements.

Principle of the Test: The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hygienic hand-rub. The procedure and protocol in this testing series matches exactly the procedures and protocols as described in 6 above. The contamination fluid in this testing series used E. coli at the very high concentration of ~108 cfu/ml. The hands of the test subjects were immersed in the contamination fluid for 5 seconds allowed to drain back and dried for 3 minutes. This testing series employed 12 healthy adult subjects.
Test Results: PASS (PBS exceeds IPA as hand-rub disinfectant)

Test Organism:	E. coli (IPA)	E. coli (PBS)
Log reduction factor (Average):	6.39	6.42
Log reduction (Average):	5.25	5.37

The above values were averaged for the 12 subjects.
Conclusion: BioGlove PBS when used neat as received can be considered a suitable hygienic hand-rub giving significantly better reduction of bacterial numbers than IPA.

Test Date: 01/07/13 EN1500 (15 subjects)
Test Material: BioGlove PBS
Test: CEN Standard EN 1500, Chemical disinfection and antiseptics- hygienic hand-rub. Principle of the Test: The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hand-rub. The procedure and protocol in this testing series was conducted at a fully accredited Medical College and Hospital (joint facility) by degreed Medical Doctors. The procedures and protocols described as follows in this testing series matches exactly the procedures and protocols as described in 6. and 8 above. This testing was conducted to verify the previous tests. It was conducted at a hospital by staff on a different continent to insure that test results were globally reproducible. The contamination fluid in this test series was again E. coli (108 cfu/ml) and administered as previously described by immersion of hands for 5 seconds. This testing series employed 15 healthy adult subjects. Test Results: PASS (PBS exceeds 60% 2-propanol as hand-rub disinfectant.

Test organism:	E. coli (IPA)	E. coli (PBS)
Log Reduction (Average)	5.27	5.32
Standard Deviation:	0.39	0.44

Number of Test Subjects: 15
Conclusion: BioGlove PBS passes the requirements of EN1500 for hygienic hand-rub when tested under conditions as described above.

The previous was performed under contract with the Vardham Mahavir Medical College & Safdarjung Hospital.

Test Date: 12/16/2011 EN- No comparable protocol exists
Test Material: BioGlove PBS
Test: Re-inoculation study after 24 hours. No Comparable EN protocol exists for this testing. Principle of the Test: This testing was devised to determine the efficacy of PBS remaining on a substrate after 24 hours by re-inoculation of bacteria. Test Procedure: Sample glass slides were coated with PBS and left to dry 1 hour in a Bio-safety cabinet. The slides were then inoculated with the organisms, left for 1 hour and enumerated and again for 24 hours before re-inoculation. Two time intervals were tested for the slides, 1 hour (no re inoculation of the organisms) and 24 hours (re-inoculation of the Staphylococcus aureus organisms) and re-inoculate positive control slides in parallel.

Test Organism:	S. aureus (Control)	S. aureus (PBS)	% Reduction
1 Hour	$4.4X10^5$	<10	99.98
24 hours	$6.2X10^5$	$6.2X10^3$	99.0

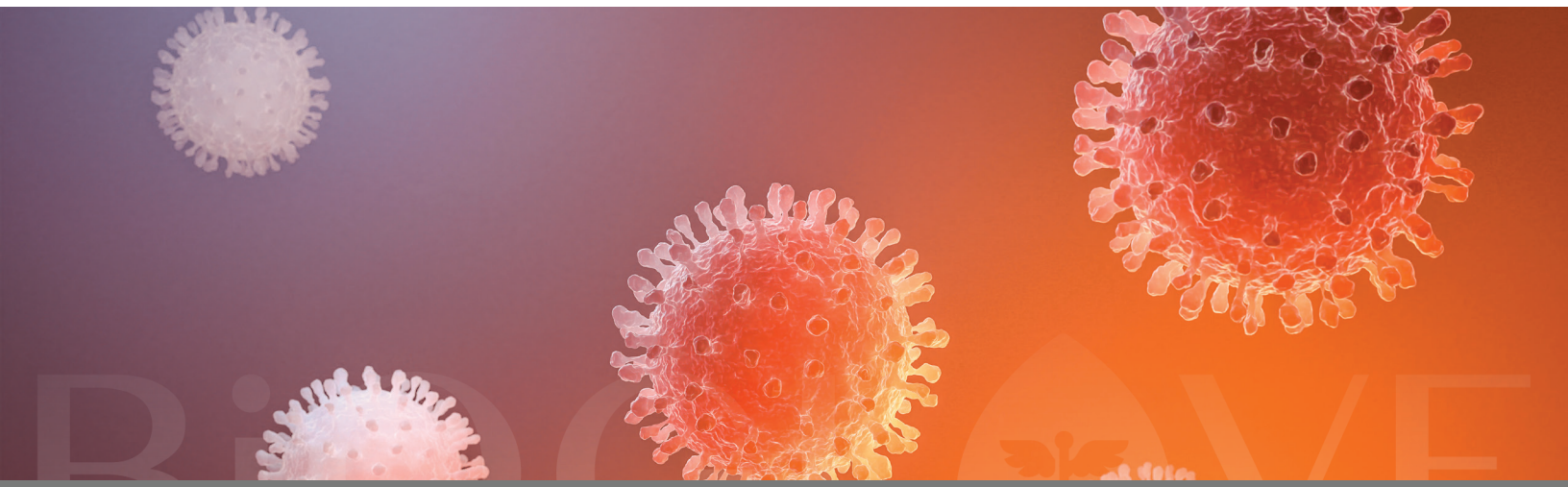
Conclusion: The results indicated that BioGlove PBS achieved greater than 4 log reduction at 1 hour of contact time against S. aureus. The results also indicated that PBS achieved 2 log reduction after 24 hours of contact time and re-inoculation against S. aureus. This indicates that PBS will remain active on the skin for 24 hours after application.

Test Date: 01/22/Test Material: PBS2010 EN14476 (Swine virus)
Test: EN14476: 2007-02, Chemical disinfectants and antiseptics- virucidal quantitative suspension test. Principle of the Test: To evaluate the virus-inactivating properties of the hand disinfectant PBS against Influenza A H1N1 using quantitative suspension assay.

Test Conditions: Testing temperature was 20 degrees C, concentration of the product was 80.0% (due to the addition of the virus strain and interfering substance, BioGlove PBS), contact time with the virus was 30 and 60 seconds. Infectivity assay was determined by means of end point titration using the micro-titre process. Investigations fro determination activity followed EN 6.6. The PBS product was examined as diluted (80%) in hard water according to EN 5.2.2.2. Contact times were 30 and 60 seconds. According to EN 14476:2007-02 a disinfectant solution at a particular concentration is having virus-inactivation efficacy if the titre is reduced by 4 log steps within the recommended exposure period. A reference test validation using formaldehyde (0.7 w/v %) according to EN 6.6.7.1 was included.
Test Result: PASS

Test Organism:	Influenza A virus H1N1
Log Reduction (30seconds):	>4.0
Log Reduction (60 seconds):	4.63
Log Reduction with formaldehyde (5 minutes):	>4.0

Conclusion: BioGlove PBS was able to inactivate Influenza A virus H1N1 (swine) in 60 seconds exposure time achieving a reduction factor of 4.63 (>99.99%).



PATHOGENS

Gram Positive Bacteria
Bacillus sp. (Vegetative cell)
Clostridium difficule
Clostridium difficule (spore)
Corynebacterium diptheriae
Listeria monocytogenes
Micrococcus lutea Micrococcus sp.
Mycobacterium tuberculosis
Mycobacterium smegmatis
Propionibacterium acnes
Staphylococcus aureus
Staphylococcus aureus (MrsA)
Staphylococcus epidermidis
Streptococcus faecalis
Streptococcus mutans
Streptococcus pneumonia
Streptococcus pyogenes

FUNGI, ALGAE, MOLD, YEAST, SPORES

Alterania alternate
Aphanizomenon sp.
Aspergillus niger
Aspergillus sydowi
Aspergillus terreus
Aspergillus versicolor
Aspergillus verrucaria
Aureobasidium pullans
Candida albicans
Candida pseudotropocalis
Chaetomium globsum
Cladosporium cladosporioides
Chlorella vulgaris
Dreschslera australiensis
Epidermophyton sp.
Gliomastix cerealis
Gloeophyllum trabeum
Microsporum sp.
Microsporum audouinii
Monilia grisea
Oscillatoria
Penicillium chrysogenum
Pencillium commune
Penicillium funiculosum
Penicillium pinophilium
Penicillium variable
Phoma fimeti
Pithomyces chartarum
Poria placenta
Scenedesmus
Saccharonyces cerevisiae
Scolecobasidium humicola
Selenastrum sp.
Trichoderma viride
Trichophyton interdigitale
Trichophyton maidson
Trichophyton mentogrophytes
Trichophyton sp.

VIRUSES

Adenvovirus Type ii & iV
Bovine Adenovirus Type i & iV
Herpes simplex Type I
Herpes simplex Type ii
Hiv - 1 (Aids)
Influenza A2 (Asian)
Influenza B
Mumps

Norovirus (Caliciviride)

Parinfluenza (sendai)
Rous sacoma
Reovirus Type I
Simian Virus 40
Vaccinia
Ms2
PrD1

PROTOZOA / PARASITES

Cryptosporidium
par vum (oocysts)

GRAM NEGATIVE BACTERIA

Acinetobacter calcoaceticus
Aeromonas hydrophilia
Citrobacter deversus
Citrobacter freundi
Enterobacter aerogenes
Enterobacter aglomerans
Enterobacter cloacae
Enterococcus
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumonia
Klebsiella terriena
Legionella pneumophila
Morganella morganii
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorscenes
Salmonella cholera susis
Salmonella typhi
Salmonella typhimunium
Serratia liquifancens
Serratia marcesens
Xanthomonas campestris

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BioGlove PBS Hand Sanitizer Efficacy Against Feline Infectious Peritonitis Virus: Hand Trials Final Report

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Objective:
Determine the efficacy of BioGlove PBS Hand Sanitizer Foam against feline infectious peritonitis virus on hands.

Methods:
Four volunteers were recruited and went through a 7 day washout (conditioning) period. During this time use of antibacterial soaps and shampoos was restricted. After the washout period volunteers will report to the laboratory for hand sanitizer testing.

The volunteers' hands were washed with a mild soap for 15 seconds and dried using a paper towel. After completing the wash, hands were seeded 3 times with 1.5mL of a high titer suspension of feline infectious peritonitis virus (a MERS-corona virus surrogate). Following final virus application hands were allowed to air dry. A before sample was collected using a sterile swab containing a neutralizing solution. Volunteers repeated the preliminary wash and seeding procedures. Following successful seeding, hand sanitizer foam was added to the volunteers' hands according to manufacturer's instruction. An after sample was then collected following the above method.

Viral samples were assayed using the tissue culture infectious dose 50% technique (TCID50) and allowed to incubate at 37°C for 14 days. Each day cultures were reviewed to determine the presence of virus. Following the 14 days of incubation the efficacy of BioGlove PBS hand sanitizer was calculated using statistical methods.

Results:
BioGlove PBS Hand Sanitizer was able to achieve a >99.9% (>3.19 log10) reduction. All replicates in the presence BioGlove PBS Hand Sanitizer Foam were below the limit of detection for the assay suggesting that greater reductions are achievable. Results are shown in Table 1.

Table 1. BioGlove PBS Hand Sanitizer Test Using Four Human Subjects Against Feline Infectious Peritonitis Virus (FIPV)

Human Subject Designation	Sample Id	Viral Titer (Log_{10} per mL)[a]	Log_{10} Reduction	Percent Reduction
Human Subject No. 1	Pre-Exposure Control	6.00	N.A.	
	Post-Exposure Test	≤ 2.50	> 3.50	> 99.97%
Human Subject No. 2	Pre-Exposure Control	5.75	N.A.	
	Post-Exposure Test	≤ 2.50	> 3.25	> 99.94%
Human Subject No. 3	Pre-Exposure Control	5.00	N.A.	
	Post-Exposure Test	≤ 2.50	> 2.50	> 99.7%
Human Subject No. 4	Pre-Exposure Control	6.00	N.A.	
	Post-Exposure Test	≤ 2.50	> 3.50	> 99.97%
Average	**Pre-Exposure Control**	**5.69**	**N.A.**	
	Post-Exposure Test	**≤ 2.50**	**>3.19**	**>99.9%**

[a] Detection Limit = ≤ 2.50 log_{10} per ml

BioGlove PBS Hand Sanitizer Foam is effective at reducing feline infectious peritonitis virus on hands by at least 99.9% following manufacturer's instruction.

Results verified by:

Associate Professor & Program Director
Environmental Health Sciences