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WHITE PAPER:
EFFICACY OF EASYDECON® ON SARS-CORONAVIRUS

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EXECUTIVE SUMMARY

- DF200, a product originally invented by Sandia National Laboratories under grant by the US Department of Energy, has been tested extensively in laboratory and field settings and shown to achieve a 3-4 log inactivation of bacteriophage virus (SARS, Corona Virus) in 30-60 seconds. It kills by a chemical oxidation/reduction reaction, and the pathogens cannot grow immune.
- DF-200 is now marketed by Intelagard under the trade name EasyDECON®.
- EasyDECON ® is inherently biodegradable and thus simplifies both application and clean-up.
- EasyDECON ® is only mildly corrosive.
- EasyDECON® can be applied using various methods, such as applied as a liquid, as a foam, and/or as a fog, depending on the circumstances of the area being decontaminated.

INTRODUCTION

The first major epidemic fueled by the Corona Virus (CoV) occurred in China in 2019. Some studies by a lab in India[(1)] found a number of portions of the virus chain which were duplicates of similar portions found on the SARS virus and the HIV virus. The areas discovered on the CoV were falsely attributed to human intervention, but this unreviewed report was discredited, and the accusations of purposeful insertions were removed.

Coronaviruses were first identified nearly 60 years ago, but they received notoriety in 2003 when one of their members was identified as the etiological agent of the Severe Acute Respiratory Syndrome (SARS-CoV). Studies on the SARS virus in 2004 led to a patent titled “Corona Virus Isolated from Humans” (2) and stated that the SARS-CoV, the newly isolated corona virus was the causative agent of SARS, and was termed SARS-CoV. Coronaviruses are positive strand RNA viruses that cause disease in humans, and domestic and companion animals. It is interesting that the CoV contains protein chains which are exactly the same chains as found in the SARS virus and the HIV virus. The Corona Viruses are similar to all viruses. See Figure 1 below:

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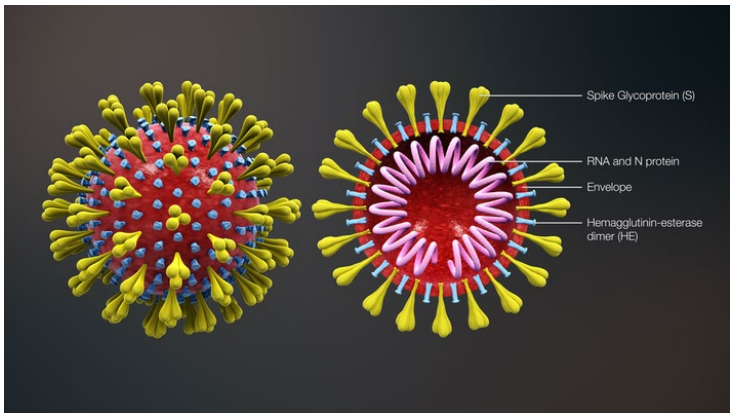

Figure 1: Structure of Corona Virus: From Corona-Live-Dashboard-Tracker, February 26. 2020

Lab studies done at that time by Sandia National Laboratories demonstrated the high effectiveness of EasyDECON® against the SARS-CoV virus[3].

We are currently experiencing another outbreak of the CoV. The epidemic is spreading rapidly, despite efforts to contain it. It has been detected on Cruise ships, Airplanes, and hotels, as well as other common places such as offices. See Figure 2 for a map of cases provided by John Hopkins CSSE on February 26, 2020:

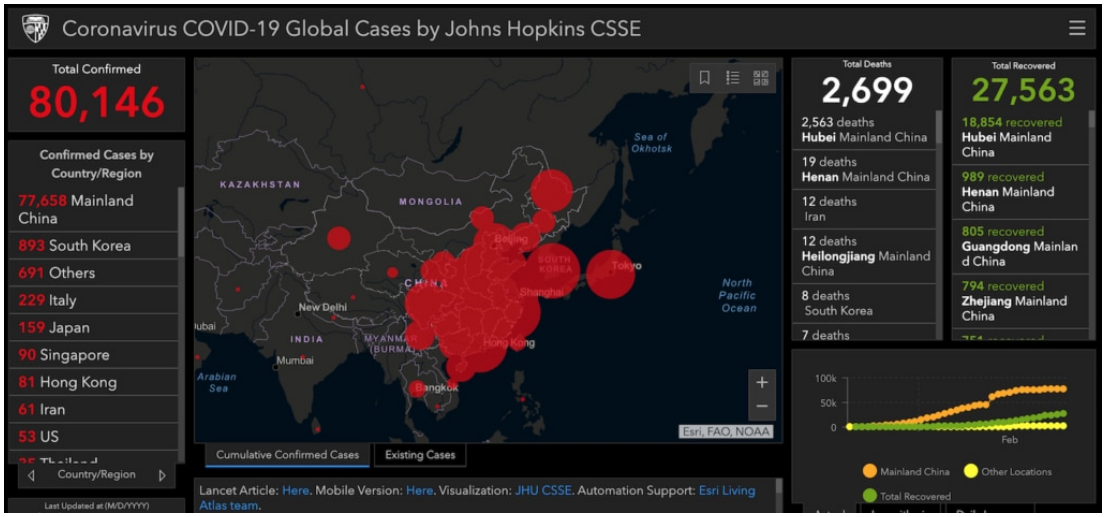

Figure 2: Growth and Spread of the Corona Virus Infections. Map shows Far Eastern areas, while numbers show total Global data.

Further, on February 26, 2020, the CDC issued a warning that it expects the Corona Virus to spread to the US and they are preparing for a potential pandemic.

DISCUSSION

Formula Development

DF-100 (Decon Formula 100) which evolved into the improved DF-200 was created by Sandia National Laboratories (Sandia) in the late 1990's. After demonstrating efficacy in numerous lab tests, it was licensed to Envirofoam Technologies, Inc. (EFT) in early 2000 and it became EasyDECON® [(4)].

The author of this paper, Mr. Robert H. Comstock, Chemical Engineer, was Director of Operations for EFT at that time and led the commercialization of DF200. This product, EasyDECON®, was subsequently deployed by the military for Desert Storm, where it provided protection for the troops and equipment during that campaign.

My Comstock continued to work with Sandia on a product enhancement, a concentrated version, the success of which led to issuance of a new patent [(5)] with Mr. Comstock listed as co-inventor.

Efficacy

1. Kill of Biological Agents

Some consider the Biological Weapon threat to be more serious than the Chemical Weapon threat. This is in part because of the high toxicity of BW agents, their ease of acquisition and production, and difficulty in detection. There are hundreds of biological warfare agents available for use by terrorists. They may be grouped into the categories of spore forming bacterium (e.g., anthrax), vegetative bacterium (e.g., plague, cholera), virus (e.g., smallpox, yellow fever), and bacterial toxins (e.g., botulism, ricin). The focus of this white paper is on the decontamination of spores as they are recognized to be the most difficult microorganism to kill.

Bacterial spores are highly resistant structures formed by certain gram-positive bacteria usually in response to stresses in their environment. The most important spore-formers are members of the genera, *Bacillus* and *Clostridium*. Spores are considerably more complex than vegetative cells. The outer surface of a spore consists of the spore coat that is typically made up of a dense layer of insoluble proteins usually containing a large number of disulfide bonds. The cortex consists of peptidoglycan, a polymer primarily made up of highly cross-

linked N-acetyglucosamine and N-acetylmuramic acid. The spore core contains normal (vegetative) cell structures such as ribosomes and a nucleoid.

Since their discovery, considerable research has been carried out to investigate methods to kill bacterial spores. Although spores are highly resistant to many common physical and chemical agents, a few antibacterial agents are also sporicidal. Many powerful bactericides, however, may only be inhibitory to spore germination or outgrowth (i.e., sporistatic) rather than sporicidal. Examples of sporicidal reagents, using relatively high concentrations, are glutaraldehyde, formaldehyde, iodine and chlorine oxyacid compounds, peroxy acids, and ethylene oxide. In general, all of these compounds are considered to be toxic.

There are several mechanisms generally recognized for spore kill. These mechanisms, which may operate singularly or simultaneously, are described below:

The dissolution or chemical disruption of the outer spore coat may allow penetration of oxidants into the interior of the spore. Several studies (King and Gould, 1969; Gould et al., 1970) suggest that the S-S (Disulfide) rich spore coat protein forms a structure which successfully masks oxidant-reactive sites. Reagents that disrupt hydrogen and S-S bonds increase the sensitivity of spores to oxidants. A typical protein with disulfide linkage is shown in Figure 3 below.

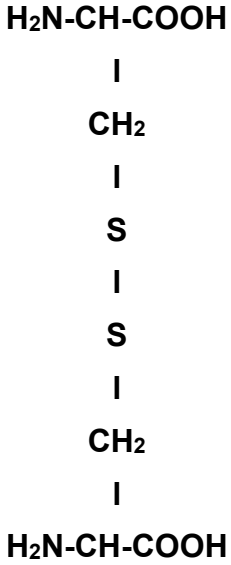

FIGURE 3: Protein with cysteine linkage.

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Table 1: Kill of Spore Forming Bacteria

		% Kill	
Agent/Simulant	**Time**	**pH 7.0**	**pH 8.0**
Anthrax Spores	30 min.	99.99	99.99
	1 hour	99.99999	99.99999
Anthrax simulant (B. Globigii spores)	30 min.	99.99	99.99
	1 hour	99.99999	99.99999

Table 2: Biological Agents Tested and Killed

EasyDECON® IS PROVEN TO NEUTRALIZE/KILL BIOLOGICAL AGENTS

Aspergillus Niger	Foot & Mouth (FMD)	Cholera
Erwiniaherbicola	Pseudomonas P.	Influenza A
Plague -Y. Pestis	Bacillus subtilis	Salmonella choleraesuis
Avian Flu H5N8	Hemorrhagic Fever (VHFs)	Citrus Canker
Escherichia Coli	Rhinovirus–Mult. Strains	Listeria monocytogenes
Pseudomonas A.	Bovine coronavirus	SEB (Staph Toxin)
Avian Flu H5N1	Hepatitis A (HAV)	Clostridium botulinum
Feline Calicivirus	Ricintoxin	Mycobacterium bovis
Pseudomonas F.	Candidabombicola	Staph A (MRSA)
Bacillus anthraces	HIV Type 1	Clostridium sporogenes
Smallpox	Salmonella enterica	Norovirus
Tularmia	Penicillium digitatum	Yellow Fever Virus

2. Testing on Bacteria and Molds

Table 3: AOAC Use Dilution Test Results*(Molds)

Lot #	Challenge Species	Results
479-110	Staphylococcus Aureus	100% 'Killed'
	Pseudomonas Aeruginosa	
	Salmonella Choraesuis	
479-114	Staphylococcus Aureus	100% 'Killed'
	Pseudomonas Aeruginosa	
	Salmonella Choraesuis	
479-112	Aspergillus Niger	100% 'Killed'
	Pencillium Digitatum	
479-113	Stachybotrus Chartarum	100% 'Killed'

*All tests used inoculated Porcelain Pennicylinders as carriers, per test protocol

3. Testing on SARS-CoV virus

Severe Acute Respiratory Syndrome (SARS) is caused by a coronavirus that remains infectious for extended periods in the environment. Research was done to evaluate the efficacy of Sandia developed decontamination formulations, such as DF-200, at various concentrations against the SARS coronavirus. SARS virus has been recently classified under antigenic group II among the family *Coronaviridae*. Bovine coronavirus (BCV) was used as safe a surrogate of SARS virus for studying the viral inactivation.

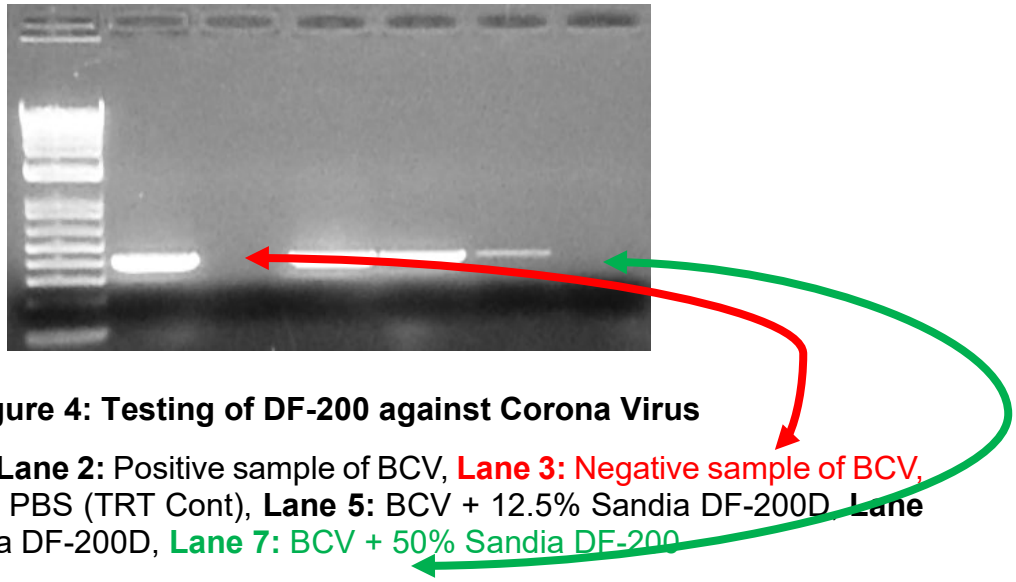

Figure 4: Testing of DF-200 against Corona Virus

Lane 1: DNA ladder, **Lane 2:** Positive sample of BCV, **Lane 3:** Negative sample of BCV, **Lane 4:** BCV + 0.1M PBS (TRT Cont), **Lane 5:** BCV + 12.5% Sandia DF-200D, **Lane 6:** BCV + 25% Sandia DF-200D, **Lane 7:** BCV + 50% Sandia DF-200

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APPLICATION METHODS-INTELAGARD EQUIPMENT

EasyDECON® can be delivered to the toxins in a variety of manners and phases to provide the necessary detoxification. One useful form of delivery is foam. A non-toxic, non-corrosive aqueous **foam** with enhanced physical stability for the rapid neutralization of toxins, especially BW agents, was the primary focus of the early development work. Sandia used Intelagard's Macaw® Backpack for foam testing. The foam formulation was based on a surfactant system to solubilize sparingly soluble toxins and to increase rates of reaction with nucleophilic reagents. The formulation also included mild oxidizing agents to neutralize biological toxins along with components to enhance the physical stability of the foam.

This neutralization technology was attractive for civilian and military applications for several reasons including: (1) a single neutralization solution could be used for both chemical and biological toxins, (2) it was rapidly deployable, (3) mitigation of agents was accomplished in bulk, aerosol, and vapor phases; (4) it exhibited minimal health and collateral damage, (5) it required minimal logistics support, (6) it had minimal run-off of fluids and no lasting environmental impact, and (7) it was relatively inexpensive.

Methods such as **sprays, mists and fogs** can also be utilized with the same basic formulation. The objective of these alternative methods is to minimize the quantity of water that is required to be used in the restoration of controlled environments (such as indoor facilities) and facilitate access of the formulation to the Bio agents.

The alternative deployment methods have various advantages over foam deployment for small or difficult area decon. In one embodiment, the formulation of the original invention was an aqueous-based formulation that was capable of being deployed as a fog (i.e., as an aerosol with particulate sizes ranging from 1-30 microns) for the rapid neutralization of target contaminants. A **fog**, can be used to achieve effective decon in areas where decontamination by a foam is difficult, if not impossible. One example is the interior of air conditioning ducts. A fog can be generated at registers and other openings in the duct and travel a significant distance inside of the duct to decontaminate hard to reach places. An additional advantage of a fog is that a relatively automated or semi-automated decontamination system can be set-up at the scene of an attack. Remotely activated foggers can be placed inside of a facility and turned on at periodic intervals (from a remote location) to completely decontaminate the facility. This method greatly decreases the potential for decontamination personnel to be exposed to a CBW agent.

The formulation exhibits low-corrosivity and low-toxicity properties and can be deployed through commercially available fog generating devices. Current decontamination formulations utilize toxic and/or corrosive chemical to achieve destruction of CBW agents that can potentially damage sensitive equipment with which it comes into contact.

Intelagard's Macaw Backpack

See the Intelagard web site to review other application equipment available, including large area spraying equipment and fogging equipment, or refer to the Intelagard Introductory Briefing[6]

RESULTS AND DISCUSSION

Exhaustive testing of EasyDECON® has shown it to be highly effective on biological agents and pathogens. It has achieved up to 7 log kill (99.99999%) even on difficult to kill spore forming bacteria. It has demonstrated complete inactivation of the corona virus simulant BCV after 1 minute of exposure with concentrations as low as 12.5% of the recommended concentration and in the presence of contaminating organic material including feces and compost.

EasyDECON® kills pathogens by a chemical oxidation/reduction reaction as opposed to waging a biological attack via antibodies; this means that pathogens cannot develop an immunity to the product.

It can be applied as a foam, liquid or fog which allows the user to select the most appropriate deployment method to be used for the area being treated.

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CONCLUSIONS

1. The EasyDECON® decontamination formulation has been proven to kill all biological agents it has been tested against.
2. The EasyDECON® decontamination formulation is deemed to be highly effective at completely inactivating SARS-like coronaviruses as demonstrated by inactivation of the Bovine Corona Virus in lab tests.
3. EasyDECON® can be applied as a foam, liquid, or fog according to the circumstances of the area to be treated.
4. EasyDECON® is only mildly corrosive.
5. EasyDECON® is inherently biodegradable.

This paper was prepared by Robert H. Comstock, Senior Chemical Engineer

Signature

February 27, 2020
Date

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