Influenza A Decontamination Experiments with Sandia DF-200 (Preliminary Results)

J. M. Bieker, G. Brown, M. D. Tucker, R. D. Oberst, and S. Kapil
Sandia National Laboratories, PO Box 5800, Albuquerque, NM 87185 (505-844-7264; mdtucke@sandia.gov)

Sandia Decontamination Formulation

Sandia National Laboratories has developed a novel decontamination formulation (DF-200) for neutralization of chemical and biological warfare agents. Due to the effectiveness of the formulation against *Bacillus* species spores, reduced concentrations of the formulation could be used against less resistant organisms including coronaviruses and the avian influenza virus. Sandia's decontamination formulation is a combination of quaternary ammonium compounds (QAC), a low concentration of hydrogen peroxide, and a novel peroxide activator and provides both detergency and disinfection capability. DF-200 was initially developed as a foam but it can also be used as a liquid spray, mist, or fog.

Inactivation of H1N1: concentration and exposure effects of various disinfectants

Studies were conducted to determine the efficacy of various disinfectants against the swine influenza A, H1N1 virus (a surrogate for the avian influenza virus). Sandia DF200D (DF-200 with reduced concentrations of the active ingredients), Bleach (sodium hypochlorite), and ethanol were all used in these experiments. To determine the concentration effect of the various test disinfectants on the swine H1N1 virus, a 1:1 ratio of virus:disinfectant was used by adding 200 µl H1N1 to 200 µl test disinfectant. The concentrations tested were as follows: 10% bleach, 1% bleach, 50% DF200D, 25% DF200D, 12.5% DF200D, and 70% ethanol ALL EXPOSED FOR 1 MINUTE. Following 1 minute exposure, samples were serially diluted tenfold by adding 100 µl to 900 µl 0.1M PBS (10^{-1}, 10^{-2}; for toxicity purposes) and infected into Madin-Darby Canine Kidney (MDCK) cells.[1] To determine the exposure effect of the various test disinfectants on the H1N1 test virus, a 1:1 ratio of virus:disinfectant was used by adding 200 µl H1N1 to 200 µl test disinfectant. The exposure effect was determined by holding the concentration of the test disinfectants constant and exposing for 1, 15, and 30 minutes. The concentrations used were as follows: 1% bleach, 12.5% DF200D, and 35% EtOH. After the specified exposure duration, samples were serially diluted once and infected into the MDCK cells in the same 96 well plate used for the concentration effect.

For the assessment of the concentration and exposure effect on the integrity of the viral RNA, the RNA was extracted from each undiluted sample and RT-PCR was conducted using the following primers targeting N1: (Forward) 5' GGT TCC AAA GGA GAC ATT TTT G 3' and (Reverse) 5' CTA TCC AAA CAC CAT TGC CAT A 3' (Integrated DNA Technologies, Inc., Coralville, IA).

Results (MDCK): Concentration Effect

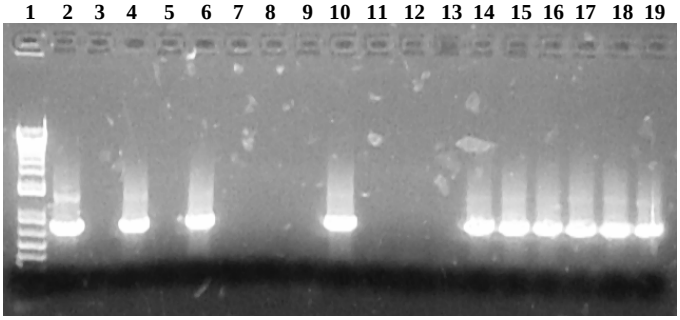
Treatment	Reaction	Durations
Positive Clinical	Positive	N/A
Negative Clinical	Negative	N/A
H1N1 + PBS	Positive	N/A
12.5% DF200d	Negative	1 min
25% DF200d	Negative	1 min
50% DF200d	Negative	1 min
1% Bleach	Negative	1 min
10% Bleach	Negative	1 min
70% Ethanol	Negative	1 min

Results (MDCK): Exposure Effect

Treatment	Reaction	Durations
Positive Clinical	Positive	N/A
Negative Clinical	Negative	N/A
H1N1 + PBS	Positive	N/A
12.5% DF200d	Negative	1 min
12.5% DF200d	Negative	15 min
12.5% DF200d	Negative	30 min
1% Bleach	Negative	1 min
1% Bleach	Negative	15 min
1% Bleach	Negative	30 min
35% Ethanol	Negative	1 min
35% Ethanol	Negative	15 min
35% Ethanol	Negative	30 min

*Results following infection into MDCK and verified by hemagglutinin assay (HA) or fluorescent antibody (FA) tests. Detection limit is not as sensitive at by RT-PCR. (Below)

Results: RT-PCR conducted with forward and reverse N1 primers:



Concentration Effect
1: 1kb DNA ladder
2: pos clin sample
3: neg clin sample
4: trt with 0.1M PBS
5: 10% bleach 1min
6: 1% bleach 1min
7: 50% DF200D 1 min
8: 25% DF200D 1 min
9: 12.5% DF200D 1 min
10: 70% EtOH

Exposure Effect
11: 12.5% DF200D 30 min
12: 12.5% DF200D 15 min
13: 12.5% DF200D 1 min
14: 1% bleach 30 min
15: 1% bleach 15 min
16: 1% bleach 1 min
17: 35% EtOH 30 min
18: 35% EtOH 15min
19: 35% EtOH 1 min

*All treatments with Sandia DF200D resulted in complete loss of infectivity and complete loss of viral RNA integrity.

Summary:

These results indicate that the Sandia National Laboratories developed DF200D is highly effective at complete inactivation of the influenza A tested in this study, swine H1N1. All experiments are conducted with additional disinfectants including bleach and ethanol. DF200D has been developed to be highly effective against a broad range of organisms, but is much more environmentally friendly due to a much more benign composition when compared to bleach (highly corrosive). Similar viral inactivation studies have been conducted with bovine coronavirus (BCV, for SARS-like virus inactivation)[2] and results show complete loss of infectivity based on cell culture in a human rectal tumor (HRT 18) cell line followed by HA and FA. Electron microscopy of treated versus untreated BCV demonstrated that treatment likely resulted in complete disruption of the lipid envelope and structural proteins (spike glycoprotein receptor, hemagglutinin receptor) as there was no visible signs of any intact coronavirus following treatment with DF200D. Recently, similar testing has been conducted using swine influenza A, H1N1 (preliminary results shown above) and will be repeated with a low pathogenic avian influenza, H5N9 upon acquisition, although similar results are expected as viruses with similar physiochemical properties react similarly to treatment with disinfectants.[3] Similarly, *Orthomyxoviridae* would be expected to react similarly to disinfectant treatment as *Coronaviridae*. The results show that the Sandia DF200D is highly effective against both of these groups of viruses, and could potentially aid in rapid and successful containment of an outbreak. Additionally, a field use verification tool is currently being developed and validated for testing an affected environment following remediation with the Sandia DF200D. Results verifying loss of viral infectivity using this tool have resulted within 2-3 hours (for sample preparation and analysis) of sample collection following remediation and are much faster than the gold standard cell culture assays.

Follow-on Work:

Follow-on work is continuing with both the swine influenza A, H1N1 virus and with the avian influenza H5N9 virus obtained from the US CDC. Sandia is also seeking collaborators for a field test to verify these results in a real-world setting.

---

[1] Meguro, H., J. D. Bryant, A. E. Torrence, P. F. Wright. 1979. Canine kidney cell line for isolation of respiratory viruses. *J. Clin. Microbiol.* **9**:175-179.

[2] Bieker, J. M., C. A. Souza, C. V. Williams, M. D. Tucker, R. D. Oberst, and S. Kapil. 2004. Rapid inactivation of SARS-like coronaviruses. *Sandia Sand Report* SAND2004-1120.

[3] Prince, H. N., D. L. Prince, and R. N. Prince. 1991. Principles of Viral Control and Transmission. In, *Disinfection, Sterilization, and Preservation.* 4th Edition. Edited by S. S. Block, Philadelphia, Lea & Febiger, pp. 411-444.