VIROFORCE OZONE PROJECT: REPORT OF TESTS IN OFFICE AND HOTEL ROOM IN APRIL 2006

OBJECTIVES:

To conduct additional tests on the antiviral efficacy of ozone gas, using the **Viroforce Prototype Generator** in conjunction with the rapid humidification apparatus, in the Dunbar office and in a Hotel room in Vancouver, making use of the assay techniques established previously.

METHODOLOGY:

The techniques were described in detail in the comprehensive report.
In addition we used the rapid humidification device supplied by Genesis Engineering, Vancouver, to provide a rapidly attainable "pulse" of high humidity in the test room.

EXPERIMENTAL DESCRIPTION & RESULTS: TEST #1 - OFFICE

This test was carried out in the Viroforce Systems office within the Dunbar Laboratory (1220 cu ft), containing office furniture, as described previously in the comprehensive report. Replicate samples of FCV (feline calicivirus) and NV (Norovirus, three different stool samples) were dried on plastic trays, as usual, and placed on top of a desk in the office. In some samples of FCV, fetal bovine serum or stool was added to determine if the presence of this organic load could affect the outcome of the treatment (Table 1).

The Viroforce prototype ozone generator was switched on, and the ozone concentration monitored continuously until the level of 25 ppm was reached. At this point the rapid humidifier was activated, resulting in a burst of water vapor, leading to a rapid elevation of relative humidity to 92 %, which was maintained for approximately 4 minutes. The RH device and the ozone generator were then switched off, and the room was allowed to "soak" or expose to high humidity for a further 15 minutes. During this period both the RH and ozone levels gradually decreased. Then the scrubber was switched on to quickly bring down the level of ozone. All apparatus was operated remotely from the outside.

When the concentration of ozone had decreased to 1 ppm (20 min), the office door was opened and the samples retrieved immediately for reconstitution and freezing. Subsequently they were assayed for infectious virus (FCV only) and qRT-PCR (quantitative real time reverse transcription polymerase chain reaction, for both viruses).

It is evident from Table 1 that substantial inactivation of FCV and NV samples was achieved, and the reduction in RT-PCR values was similar, indicating that infectivity of both viruses would be similarly affected if it were possible to assay for NV infectivity. Conceivably, an even higher level of humidity, such as that (96%) achieved in test #2, below, might have led to greater inactivation.

In addition the presence of added serum and stool (from a NV-positive sample) did not adversely affect the response of FCV to ozone treatment.

TABLE 1., Field Test in Office

Virus	PFU (fraction of control)	Log_{10}	RT-PCR (fraction of control)	Log_{10}
FCV, no serum FCV, + FBS (1:1) FCV, + stool (1:1)	0.012 0.017 0.015	- 1.92 -1.77 -1.82	0.029 0.021 0.020	- 1.54 -1.68 -1.70
Norovirus (NV)sample 1	_		0.070	- 1.15
NV sample 2	_		0.055	- 1.26
NV sample 3	_		0.046	- 1.34

Replicate samples of 200 μL were dried onto plastic surfaces, as usual. Some of the FCV samples were first diluted 1:1 with either FBS (fetal bovine serum) or NV-positive stool. All FCV samples were placed in the same location within the Dunbar office (1220 cu ft) (on top of a desk). The NV samples were all virus positive according to RT-PCR tests; these were also placed at the same location as the FCV.
Maximum level of ozone achieved was 30 ppm, and maximum RH was 92%. The temperature did not exceed 24 ºC.
Control values for FCV infectivity, 5.1×10^4 pfu/mL; and for PCR, ranged from 116 to 218 ng RNA; for NV samples, NV 1 = 58.15 ng RNA, NV 2 = 129.5 ng RNA, NV 3 = 114.1 ng RNA.

EXPERIMENTAL DESCRIPTION & RESULTS: TEST #2 – HOTEL ROOM

This test was carried out in a typical hotel room (1732 cu ft), containing the main room with double bed and table and chair, open closet, and bathroom containing bath/shower and sink and toilet. The single window contained an air conditioning unit, which we taped to seal cracks. We also taped closed the ventilation outlet.

Replicate samples of FCV were prepared at the Dunbar Laboratory on plastic trays, as usual (3 trays containing duplicate spots of virus), and transported in sterile containers to the hotel room. They were placed in 3 different locations, one in the bathroom next to the sink, one on top of the bed, and the third on top of the table. The Viroforce Prototype ozone generator and rapid humidifier were placed centrally in the room, and the ozone monitor and controls were operated by remote control from the corridor. The sequence of controls and timing were essentially the same as for test #1.

After the treatment, when the ozone level had decreased to less than 1 ppm, the room door was opened and the samples retrieved for transport back to Dunbar Laboratory. Control dry virus samples had been left within the biosafety cabinet at the Dunbar Lab. The total duration of the experiment, from leaving Dunbar Lab and returning to the Lab, was 4 hours. The data for this experiment are summarized in Table 2.

Samples were subsequently assayed for virus infectivity and qRT-PCR, as usual. In the case of FCV samples that gave no virus plaques at 1:10 dilution (bathroom and table samples), these were re-assayed by the cpe end-point dilution test, to determine if there were a few remaining infectious viruses left after treatment. Control and bed samples were also assayed for comparison. Since the bathroom and table samples gave no cpe in these assays, we concluded that for these samples the virus had been eradicated.

TABLE 2. Field Test in Hotel Room:

Virus	PFU (fraction of control)	Log_{10}	RT-PCR (fraction of control)	Log_{10}
FCV, bathroom	0[a]	<- 4.0	0.077	- 1.11
FCV, bed	< 0.0002	<- 3.7	0.077	- 1.11
FCV, table	0[a]	<- 4.0	0.075	- 1.12

Replicate samples (200 μL) of FCV were dried onto plastic surfaces in the Dunbar Lab as usual, and transported to the Hotel room, capacity 1732 cu ft. containing standard furniture. Maximum ozone concentration achieved, after 25 min exposure, was 25 ppm, and after switching on the rapid humidifier for 4 min the maximum RH was 96 %. Following further exposure for 15 min the scrubber was turned on, which brought the ozone and RH values back to ambient levels within 20 min.
Control values: FCV infectivity, 8.0 x 10^4 pfu/mL, 415.5 ng RNA.

[a] These samples showed no evidence of FCV cytopathic effects when aliquots were inoculated directly into FK monolayers, in the cpe-endpoint dilution assay. They were therefore totally free of infectious FCV. The samples from the “bed” did show cpe at a dilution of 1:4, but not at 1:8. Control unexposed virus showed cpe at dilutions of at least 1:4,096.

CONCLUSIONS

1. With the aid of the Rapid Humidifier device we were able to attain the desired high levels of humidity (92 - 96 % RH) within the office and the hotel room.
2. Under these optimal conditions we obtained substantial inactivation of both feline calicivirus and Norovirus, as determined by a combination of infectivity and qRT-PCR assays.
3. In the Hotel room test we eradicated FCV in two of the three pairs of samples, and reduced the level in the other samples to fewer than 10 infectious virus particles. Thus inactivation of approximately 4 log's was achieved
4. All three samples of Norovirus (different stool samples) in the Office test were reduced to the same level, which corresponded to the similar reduction in FCV infectivity.
5. The comparison of values between FCV and NV RT-PCR confirms that these two viruses are equally susceptible to the ozone treatments. Consequently we would expect that NV infectivity could also be reduced by 4 log's in the Hotel room conditions.
6. Samples of FCV mixed with bovine serum, and with stool from a NV-positive sample, were equally susceptible to the ozone/humidity treatment. Thus the presence of an organic load did not affect the susceptibility of virus to the treatment.