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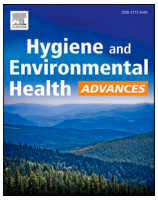

The influence of environmental conditions and hypochlorous acid (HOCl) fogging on the infectivity of H1N1 influenza virus

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ABSTRACT

The influence of different environmental factors on the infectivity of H1N1 influenza virus was measured. We found less than 1-log reduction when viruses were exposed to NaCl solutions up to 5 M or solutions with pH = 4 through 11 for one hour. The infectivity of the virus was sensitive to the solution temperature. A log reduction ranging from 2 to more than 6 was observed starting at 50 °C and spanning just 3 or 5 for 10- or 30 min exposures, respectively. Drying the virus in media on aluminum or polymer coupons for 48 h resulted in a log reduction of 4 when kept at room temperature, but less than one when kept at 4 °C or -20 °C. Log reduction greater than 6 occurred at room temperature after 3 days, but only 1-log reduction occurred at 4 and -20 °C after 6 days. Drying on different coinage achieved similar results, except for pennies, where more than 6-log reduction was observed after 24 h. HOCl was used to fog the aluminum and polymer surfaces, placed vertically, for 5 and 7.5 min to achieve a log reduction of 4 and for 6.5 respectively. Storage of opened solution containers for 9 months at ambient resulted in a decrease in chlorine concentration from 550 ppm to 240 ppm. Fogging with the old solution was still able to achieve a log reduction of 6.5 after 7.5 min fogging. The study indicates that exposure to common environmental conditions such as a wide pH range, high salinity, and low temperatures has only a minor effect on infectivity. which can persist for more than 5 days when dried on commonly encountered surfaces, allowing accumulation of infectious viral titre. Fogging with HOCl is an effective method of delivering disinfectants to large areas, achieving complete reduction of the viral titer on both horizontal and vertical surfaces.

1. Introduction

The recent COVID-19 pandemic underscored the importance of effective and efficient sterilization to control spread of viral disease. Microbes can last on different surfaces for variable periods of time (Scott and Bloomfield, 1990; van Doremalen et al., 2020). For example, COVID-19 is detectable for up to 3 h in surface aerosols, for up to 24 h on cardboard, and for up to 2 to 3 days on plastic and stainless steel (Block and Rowan, 2020). Common ways viruses spread are through touch, saliva or air (Corstjens et al., 2016; Koizumi et al., 2020; Onakpoya et al., 2021). An individual infected with influenza is able spread the virus through respiratory aerosols while breathing, speaking, coughing, or sneezing. While aerosols definitely infect more directly while they are airborne, and enter the respiratory tract of individuals (Hinds and Zhu, 2022; Sotiriou et al., 2008), the larger particles can settle on adjacent surfaces, where they can accumulate and eventually build up enough particles to transmit infection upon contact (Chu et al., 2022; Meyerowitz et al., 2021). The CDC notes that in this case, the dose of virus must be sufficient to cause infection through mucous membrane route, which in turn depends on the time the virus survives on the surface, and the rate at which its deposited. (CDC, 2023) In public areas, with a lot of traffic, deposition on surfaces sufficient to cause infection is quite probable. But the rate of accumulation will depend on other conditions, such as the material nature of the surface, temperature, pH, and salinity.

Therefore, knowledge of the factors affecting viral accumulation on surfaces together with the optimal method for disinfection of high-

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volume public areas like health care facilities is absolutely essential. We therefore chose to study in this manuscript in greater detail the influence of the surface materials, the temperature, pH, and salinity, as well as the optimal method of application of the disinfectant.

Recently, the US Environmental Protection Agency approved the use of hypochlorous acid (HOCl) against COVID-19 (Us Epa, 2020). Potential viral targets are the viral envelope, which contains lipids and is a typical membrane unit, the capsid, which is principally protein in nature (McDonnell and Russell, 1999; Wang et al., 2007). The mechanism of disinfection involves destruction of the cell wall of microbes or the capsid of viruses, allowing the disinfectant to destroy or inactivate them (Hagbom et al., 2015; Hawkins and Davies, 2002).

Hypochlorous acid was chosen as it has a history of being non toxic. Along with this, it is non-corrosive and inexpensive (Fraise, 1999). HOCl is the active ingredient in numerous mouth rinses and does not cause any detrimental systemic side effects. The antiviral properties are dependent on its concentration, duration of exposure, and state that it is being used in. The active ingredient of HOCl, also known as the available free chlorine is OCl^- and is quantified in parts per million (ppm). 1 ppm is equivalent to 1 mg/L. HOCl is a strong oxidizer when it dissociates into H^+ and OCl^- in aqueous solution. This causes denaturation and aggregation of proteins. HOCl also destroys viruses by chlorination, forming chloramines and nitrogen centered radicals. This results in single, as well as double-stranded DNA breaks, rendering the nucleic acid useless and the virus harmless (Eryilmaz and Palabiyik, 2013; Guan et al., 2023; Marcinkiewicz et al., 2000; Wang et al., 2007; Winter et al., 2008). HOCl can decontaminate surfaces carrying norovirus and other enteric viruses within 1 min at a concentration of 200 ppm. (Block and Rowan, 2020) When diluted 10-fold, HOCl solutions at 20 ppm were still effective in decontaminating environmental surfaces carrying viruses after a 10 min contact time (Park et al., 2007). Avian influenza (H5NI "bird flu") was eradicated in 10 s or less in a 2015 study (Hakim et al., 2015).

These experiments were all done by placing HOCl liquid droplets in direct contact with contaminated surfaces. Another method for disinfection is fogging, which is done by loading the liquid into an ultrasonic nebulizer, which creates a fog composed of micron sized droplets (Burfoot et al., 1999; Clark et al., 2006; McDonnell, 2006; Park et al., 2007). Fogging affords a simple way of introducing the disinfectant over large areas. HOCl dispersion with a fogger allows the disinfectant to come into contact with various contaminated surfaces at different angles, which is important for complete sterilization. It was previously shown that fogging was very effective against E. faecalis, a microorganism which is resistant to many chemical disinfectants, and remained chemically stable when the pH was maintained between 3 and 6 (Feng et al., 2022). However, the effectiveness of reducing of viral infectivity with HOCl applied to surfaces via a fogger has not been reported. Here we first explore the conditions which affect viral infectivity both in media as well as dried on surfaces and then quantify the reduction of infectivity when fogging is performed using different protocols. We chose H1N1 virus as our model system, since it is a common infectious pathogen, with multiple similarities in structure as well as method of transmission to SARS-CoV-2, and has been the source of previous pandemics.

2. Materials and methods

2.1. Sample preparation for drying and fogging test

Influenza A virus (H1N1) strain A/PR/8/34 (ATCC® VR-95™) was grown and stored in MEM Alpha (gibco) with 0.2% Bovine Serum Albumin (BSA) (Sigma). 50 μL droplets containing approximately 10^7 pfu H1N1 in PBS was smeared onto 2 cm^2 sterilized aluminum (Aluminum 2024-T3) and polystyrene (Mw = 280 k, Sigma) plates, situated in P35 petri dishes (Sarstedt) and allowed to dry in biosafety cabinet at room temperature for 1 h.

For the drying test, the P35 petri dishes were kept at -20 °C, 4 °C and room temperature for different times, up to 120 h. For the fogging test, the plates were attached in either horizontal or vertical positions in a glass vacuum bell jar where the HOCl fog was attached into the top inlet port (Fig. 1).

2.2. Sample preparation for saline and pH test

For the saline test, H1N1 viruses (~10^7pfu) were added into virus media (MEM Alpha with 0.2% BSA) with different saline concentrations. For the pH test, H1N1 viruses (~10^7pfu) were added into virus media at different pH values, which were adjusted with hydrogen chloride and sodium hydroxide. The pH values were measured after the virus added. The virus was treated for 1 h and then the virus solutions were collected for plaque assay quantification.

2.3. Sample preparation for temperature test

1 ml of H1N1 virus (~10^7pfu) was add into Eppendorf tubes and placed into a water bath (ANOVA) at different temperatures and for different time periods. After treatment, the virus solutions were collected for plaque assay quantification.

2.4. Fogging processes

The experimental setup is shown in Fig. 1A. Samples were placed in a large desiccator (VWR type 250) in horizontal and vertical positions, as shown. A tube from the fogging machine was then attached to the top of the desiccator. Hypochlorous acid eFFectant, provided by EcoLogic Solutions, was pumped into the fogging machine (Contronics HU-45 Humidifier) to generate HOCl fog and fog was allowed to fill the desiccator. Fogging was performed at a fixed air flow rate of 1000 $inch^3$/s, which contained a total volume of 30 mL of HOCl every minute in either pulsed or continuous modes. Two methods of fogging were attempted. The different protocols used are shown in Fig. 1B. The chlorine concentration was determined by titration using a Total Chlorine Test Kit (HACH) and the pH was measured using a Mettler Toledo FiveEasy F20 pH meter.

2.5. Virus quantification

MDCK2 (NBL-2) (ATCC® CCL-34™) were used for virus plaque assay to quantify the plaque forming unit (pfu). MDCK2 cells were cultured in 6 or 12 well plates to reach 90–95% confluence before adding the virus. The growth medium for MDCK2 cells is OptiPRO™ SFM (gibco) with 1X GlutaMAX (gibco) and 1% penstrep (gibco) Table 1.

Test and control samples, were assessed for the number of surviving viruses. Each sample was immersed individually in a 50 ml test tube containing 2.5 mL of MEM alpha with 0.2% BSA, then vortexed for 1 min to suspend the virus. Then, seven 1 in 10 dilutions, in MEM Alpha, were made and 0.25 mL of each dilution was added, in triplicate, into MDCK2 plates. In order to determine the lowest concentration of remaining viruses, 2.25 ml of the original virus solution was divided into three and added into three MDCK2 wells. The plates were put on the rocker for 30 min at room temperature and incubated in 37 °C 5% CO_2 incubator for 1 h. After 1 h incubation, the viruses were removed and replaced with semi-solid medium which contained a quarter volume of 1.2% tragacanth gum, a quarter volume of 2X MEM Alpha, 0.2% BSA, 1% TrypLE Select (gibco) and half volume of MEM Alpha. The plates were then incubated for 72 h at 33 °C 5% CO_2. After incubation, the plates were rinsed and stained with 1% crystal violet, 3.7% formaldehyde and 50% methanol. The plaques on dilution plates containing 5–50 plaque forming units (PFU's) were counted. When taking into consideration the inoculum dilution added into counted plates, the average of plaques on the control and test samples was calculated. The number of surviving fogged virus pfu was reported, relative to the control sample, as the log-reduction of pfu caused by the fogging treatment Fig. 2.

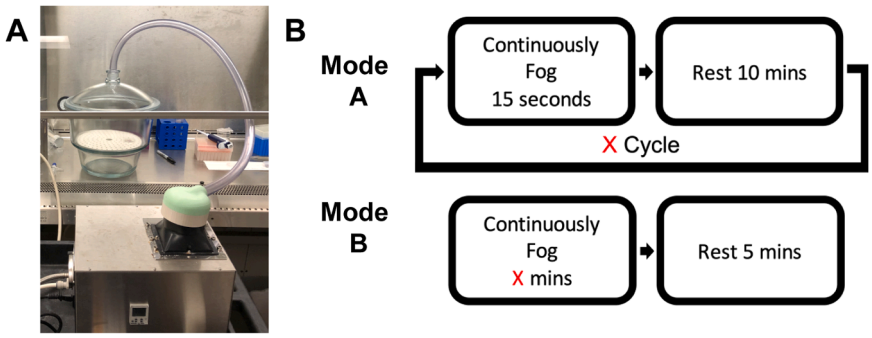

Fig. 1. (A) Experiment setup. (B) Different fogging protocols.

Table 1
Semi-solid medium content.

	2X MEM Alpha	Tragacanth Gum	MEM Alpha+0.4% BSA+1% Tryple Select
Ratio	1	1	2

2.6. HOCl solutions

eFFectant, obtained from EcoLogic Solutions, is a concentrated solution that can be ready to use or diluted. The solutions were stored in separate opaque bottles away from sunlight and then equal volumes were added into the fogging machine for testing. The same testing protocol was used in each case. The Chlorine concentration and pH were measured each time the solutions were used and the values are tabulated in Table 3.

3. Result

3.1. Temperature test

In Fig. 3A we plot the results of the plaque assays after the virus solution was kept at different temperature and different times. From the

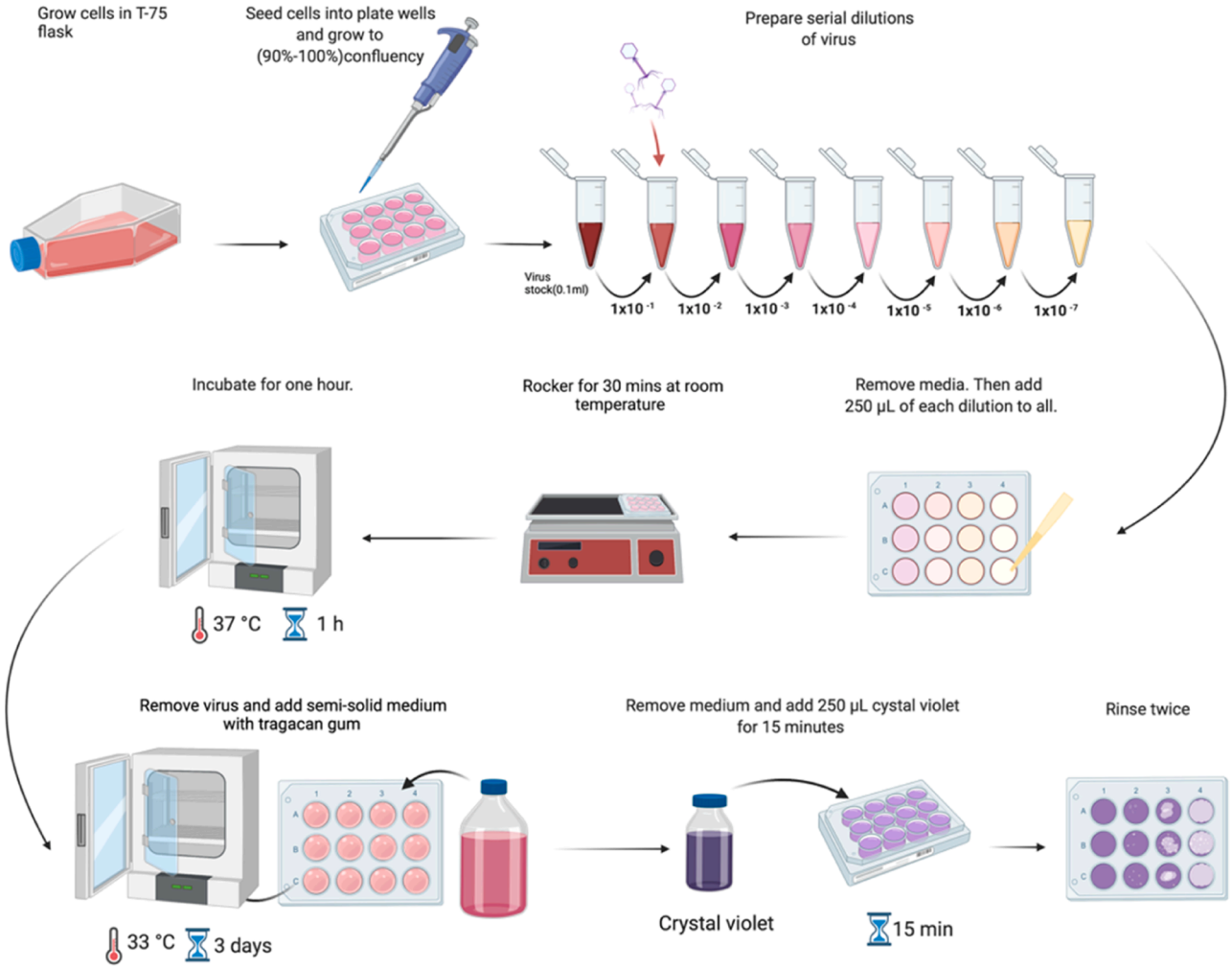

Fig. 2. Plaque assay protocol.

figure we see that minimal differences are observed between 37 and 45 °C, while a rapid decrease occurred between 45 and 55 °C, and the functional form of the reduction was dependent on the incubation time. In Fig. 3B we repeated the measurements for 10 min and 30 min within the region of 50 to 55 °C with higher precision. From the figure we can see that at 50 °C incubations of 10 and 30 min yield the same 2-log reduction. Increasing the temperature another two degrees, yields a 5-log reduction after 30 min and only a 3-log reduction after 10 min, hence as the temperature is increased the inactivation of the virus becomes more efficient at a given temperature. In either case, almost complete or more than 6-log reduction, were measured for temperatures greater than 55 °C or greater than 53 °C after only 10 and 30 min, respectively.

3.2. TEM images

In Fig. 4 we show TEM images of the wild type H1N1 virus and viral particles after being heated to 56 °C for 30 min. From the figure we can see that the wild type virus is approximately 140 nm in diameter, while the heated virus has shrunk to 50 nm in diameter. Furthermore, most of the spike proteins which are critical for the infectivity appear to have fallen off the main structure.

3.3. Saline test

Since viral particles are initially carried in saline containing fomites, and salt water is commonly used as a bacterial disinfectant (Wang et al., 2020), we also measured the influence of salt concentration on viral infectivity. Virus particles were placed in solutions with different NaCl concentration ranging from physiological, 0.25 M to 5.0 M, for 1 h. The infectivity was then measured directly using the plaque assay. The data are plotted in Fig. 5, where we can see that less than one LRV occurs even for concentrations as high as 5.0 M. ($p << 0.01$)

3.4. Test

The result of pH test is plotted in Fig. 6. From the figure, the viruses were not sensitive in the range of pH 4 to 11. However, a sharply log reduction occurred when the pH is lower than 4 or higher than 11.3.

3.5. Drying test

In Fig. 7 we plot the results as a function of drying time of plaque assay analysis from samples of media containing virus, placed on PLA, PS and aluminum plates, and allowed to dry for different times under different conditions. From the figure we can see that only small differences were observed between different materials. On average, when drying at room temperature, only a 4-log reduction in viral content was obtained after 50 microliters of H1N1 solution was allowed to dry for 48 h. If the samples were stored after drying at 4 °C, the typical temperature in a refrigerator, only a 1-log reduction was observed at 48 h and more than 4% of the virus survived after 120 h. Moreover, if the temperature was reduced further to -20 °C, the amount of infectious virus was reduced by only 95% after 48 h, with no further reduction after 120 h.

In Fig. 8 we plot the log reduction that was obtained when virus was

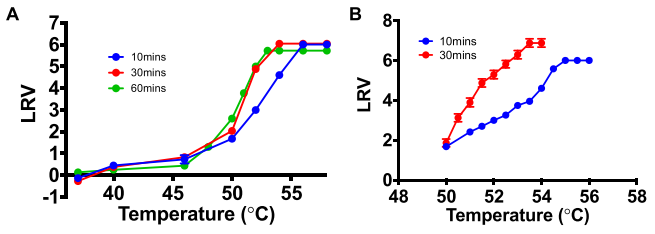

Fig. 3. Temperature test. H1N1 viruses are sensitive between 45 and 55 °C.

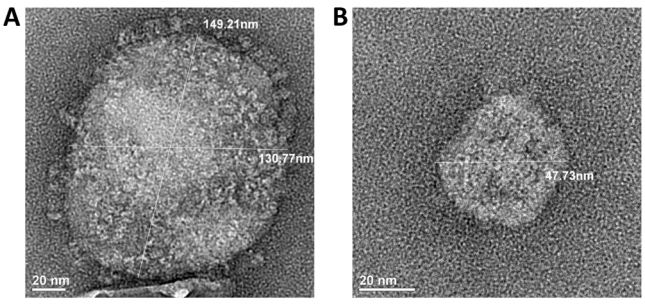

Fig. 4. TEM images. (A) H1N1 virus before treatment. (B) After treated at 56 °C for 30 min, H1N1 virus shrank from 140 nm to 50 nm and no spike proteins are observed on the main structure.

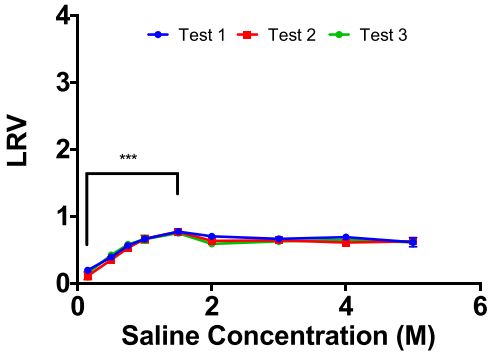

Fig. 5. Fraction of infectious virus surviving as a function of concentration after exposure to saline solution.

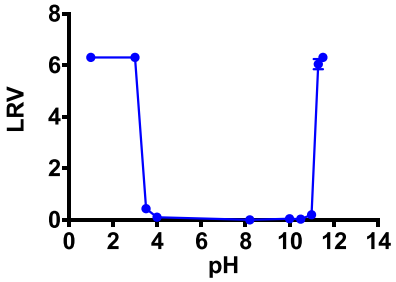

Fig. 6. pH test. The viruses were not affected between pH 4 to 11.

dried on common coinage currency, of pennies, nickels, dimes and quarters for one and 24 h at ambient conditions. After 1 h, no significant reduction was observed on any of the coins. while less than a 2-log reduction were observed on the other coins even after 24 h. In contrast nearly complete reduction of infectivity was observed on pennies after 24 h. Since the virus comes in contact with only the surface of the coins, the composition of metals in the near surface regions was also measured and compared with the compositions obtained from the Mint (Meredith, 2016). The values measured using plasma elemental Analyzer (KEYENCE VHX-7000) shown in Table 2.

3.6. Characterization of HOCl solutions

The chlorine content and pH of the HOCl solutions were tracked and tested. All bottles were stored in our laboratory at ambient conditions. We first compared the degradation rate in three bottles, from different batches, where we measured the chlorine content immediately after opening and sampled it numerous times for a period of 9 months. The data is plotted in Fig. 9A, where we can see that the rate if decay of the chlorine content appears similar in all the bottles, and the chlorine

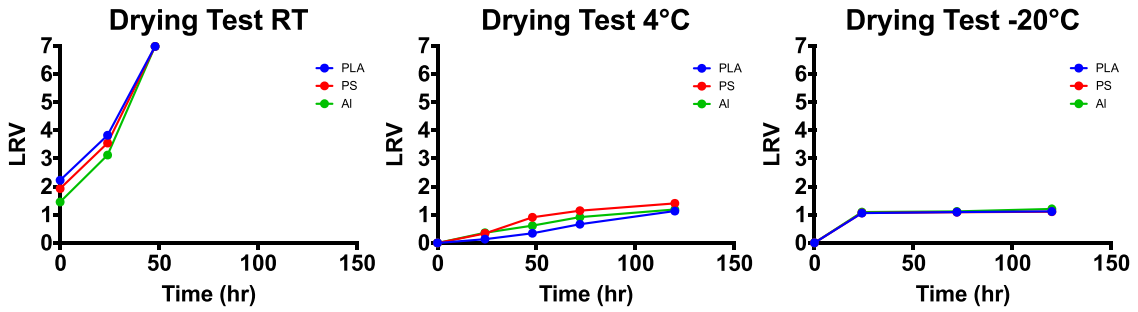

Fig. 7. Drying test. Only 4-log reduction at room temperature, 1-log reduction at 4 and -20 °C after 48 h.

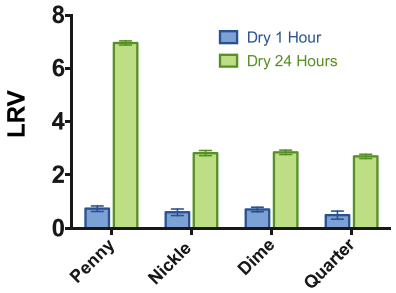

Fig. 8. Drying test on coinage. Only 2-log reduction were observed on the other coins after drying at room temperature for 24 h, except for pennies.

Table 2
Coinage Composition (Meredith, 2016).

Denomination	Clad	Total Composition (%)	Clad/ Surface Composition (wt %)
Penny	O	2.5 Cu Balance Zn	100% Cu
Nickel	X	25 Ni Balance Cu	71.9% Cu/ 27.2% Ni
Dime	O	8.33 Ni Balance Cu	71.8% Cu/ 27.2% Ni
Quarter	O	8.33 Ni Balance Cu	70.6% Cu/ 29.4% Ni

content decreases about 30% after the first hundred days in all cases. We also compared the decay of the chlorine content after 9 months with a bottle that had remained factory sealed, and chlorine content is shown in Fig. 9B, where we can see that the sealed bottle retained nearly 75% of its original concentration, as compared to the open bottle, which retained only 25%.

The stability of HOCl solutions is strongly dependent on their pH. HOCl is most stable against decomposition in the pH range of 3.5 to 5.5. At high pH HOCl rapidly decomposes to form -OCl (aq), also known as bleach, and which is highly toxic to cells and corrosive to materials. At lower pH values HOCl reacts forming chlorine gas and thereby rapidly losing its chlorine content. We therefore also measured the pH together with the associated chlorine content at different times, and the results are tabulated in Table 3. From the table we find that the pH was maintained within the range of 4.75–5.18, which is well within the range of stability for its disinfectant properties (Del Rosso and Bhatia, 2018; Pelgrift and Friedman, 2013).

3.7. Optimizing the fogging process

Two fogging methods were attempted. The aluminum plates containing H1N1 after drying for 1 h were attached in either horizontal or vertical positions (Fig. 1) in a glass desiccator where the HOCl fog was attached into the top inlet port. The plates were fogged with either mode A or B. Following exposure, the viruses were collected in virus medium and counted using plaque assay.

From Table 4, we found that horizontal sample needed 1 cycle of mode A to eliminate the H1N1 viruses. Vertical samples required 2 cycles of mode A to eliminate the H1N1 viruses. Continuously fogging samples need 5 min fogging (5 min resting) to obtain more than 4-log reductions. The overall results, tabulated in Table 4, were similar to those previously reported by Burfoot et al. (1999).

3.8. Fogging on different materials

We then focused on different materials commonly found in medical offices, we applied the same experiment setup using procedure A to

Table 3
Chlorine concentration and the pH.

	Chlorine Concentration	pH
EcoLogic Solutions	512ppm	5.03
	402ppm	5.18
	343ppm	4.82
	290ppm	4.75
	240ppm	4.96

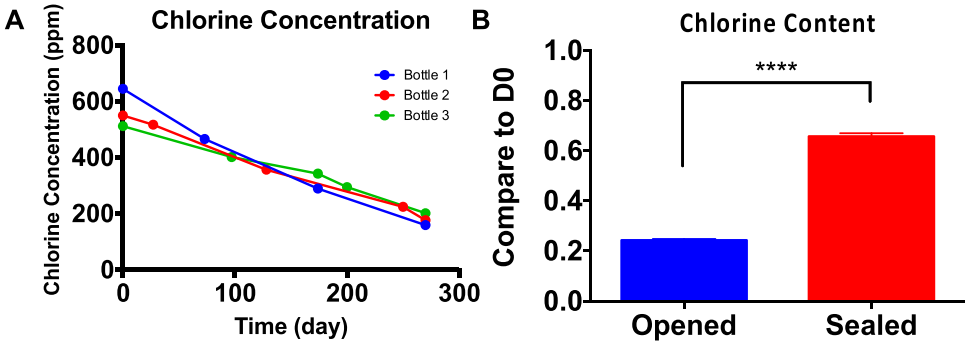

Fig. 9. HOCl solution characterization. (A) Chlorine concentration decreased around 30% after 100 days; (B) Chlorine concentration was higher in the sealed bottles after 270 days.

Table 4
Fogging results with different fogging protocols.

Mode	Orientation	X Cycles	Control	Fogged	Log Reduction
Mode A	Vertical	1	8.6×10^7	4.3×10^3	4.30
		2	1.27×10^9	0	9.10
	Horizontal	1	1.27×10^9	0	9.10
Mode B	Orientation	X Mins	Control	Fogged	Log Reduction
	Vertical	2	4.33×10^8	5.33×10^5	2.91
		5	4.33×10^8	1.10×10^3	4.90

molded polystyrene (PS) plates and aluminum plates. Even though the polystyrene samples had lower log reductions than aluminum, 5 min of continuous fog and 5 min rest were enough for both samples to reach 4-log reduction Table 5.

3.9. Degraded HOCl fogging test

In Fig. 9 above we had plotted the HOCl chlorine concentration with storage time. Here we investigated the antiviral efficacy of the HOCl on aluminum plates with different chlorine concentration, as tabulated in Table 6, the log reduction corresponding to fogging with the solutions having 550 and 240 ppm chlorine concentration. From the table we find that in the freshly opened bottle we obtained 4.08 log reductions after 5 min continuous fogging and 5 min rest while 2.81 log reductions were obtained by fogging with the same protocol using an older HOCl (240 ppm). Lower concentration of HOCl need 7.5 min continuous fogging and 5 min rest to eliminate the H1N1 viruses. These results indicate that a significant reduction in free chlorine could be tolerated and still obtain an adequate reduction in pfu's.

4. Discussion

The SARS-COV-2 pandemic has raised concerns regarding the survival of encapsulated viruses on surfaces. Here we report on a detailed study of H1N1, another encapsulated virus which was the pathogen involved in pandemics of 1918 and 2009 (Cascella et al., 2023). Previous studies showed that pH, temperature, and salinity, at levels normally encountered in nature, could impact the ability of avian influenza viruses to remain infective in water (Brown et al., 2009, 2007) and the ability of Influenza A viruses transmission (Labadie et al., 2018).

4.1. Influence of natural environmental factors

Heat appeared to be the most effective environmental factor in reducing the viral titer. Heating the virus for as little as 10 min at 55 °C resulted in a 6-log reduction in infectious titer. The acute sensitivity to temperature was also observed when the temperature was decreased by only 1.5°, the time to obtain a similar log reduction increased 30 min at the lower temperature (Fig. 3).

TEM images of the viral particles, heated for 30 min at 56 °C (Fig. 4) show that increased temperatures produce a large amount of damage to the outer envelope. The diameter shrinks by 65% and the surrounding "spike" proteins are now missing, making the viral particles incapable of infection.

Exposing the viral particles, on the other hand to high salt concentrations, up to 5 M, only reduced the infectivity by less than one log or roughly 80%, indicating that in contrast to anti-bacterial activity (Wang et al., 2020), high saline content, such as that in sea water (0.6 M) or in salt water pools, is not an effective anti-viral agent. This phenomenon was also observed for SARS-COV-2 viral particles, where theoretical modeling showed that it was due to salt bridges being formed on the proteins which stabilized the structures (Feng et al., 2023). Molecular imprinting confirmed the preservation of structure for the H1N1 virus as well (Lee et al., 2022). Fomites carrying viral particles initially contain physiological concentrations of saline, but when drying on surfaces, the saline concentration can increase significantly. These results also indicate that this increase does not affect infectivity enabling viruses to survive for extended periods after drying on surfaces.

Table 5
Fogging results on different surface.

Time (min)	Material	Control	Fogged	Log Reduction
Fog 2, Rest 5	Aluminum	4.33×10^8	5.33×10^5	2.91
	Polystyrene	8.67×10^7	7.00×10^5	2.09
Fog 5, Rest 5	Aluminum	4.33×10^8	1.10×10^3	4.90
	Polystyrene	8.10×10^7	4.12×10^3	4.29

Table 6
Fogging results with different chlorine concentration.

Time (min)	Chlorine	Control	Fogged	Log Reduction
Fog 5, Rest 5	High (550 ppm)	6.67×10^6	5.67×10^2	4.08
	Low (240 ppm)		1.07×10^4	2.81
Fog 7.5, Rest 5	High (550 ppm)	3.30×10^6	0	6.52
	Low (240 ppm)		0	6.52

The virus also seemed to be stable when immersed for 1 hour in solutions covering pH range from 4 to 11. A dramatic decrease in infectivity occurs with just small changes at either extreme where infectivity is reduced by 6 logs. A similar finding was reported for SARS-CoV-2, indicating that extreme pH must be affecting the envelope as well (Chan et al., 2020). The exact mechanism is currently being investigated via theoretical modeling of the spike protein structures.

Survival of dried viral titers on surfaces also seemed to depend strongly on temperature. The LRV vs. time data showed less than 2-log reduction after 5 days, in the temperature range between 4 to -20 °C, illustrating the dramatic stability of the virus at lower temperatures. Since this temperature range is typical of winter in the northern hemisphere, this data may also partially explain the larger occurrence of infection during the winter of "flu season". If the coupons were stored at -20 °C, an initial reduction of only 1-log was observed even after 5 days indicating that the virus is well preserved at temperatures commonly found in commercial freezers. Taken together, these results indicate that temperature has the most dramatic effect preservation of viral infectivity for long times. Hence during cold weather, and in populated areas, large viral titers can easily accumulate on surfaces and spread infection. Similarly viral titer can also accumulate in food processing plants, where products are refrigerated or frozen, in agreement with (Bailey et al., 2022) who directly studied viral propagation in the meat packing industry

4.2. Survival of virus on coinage

When virus was dried on common coinage currency, nearly complete reduction of infectivity was observed on copper pennies after 24 h, while less than 3-log reduction were observed on the other surfaces.

According to United State Mint (Meredith, 2016), quarters and dimes are cladded coins which are composed of a copper core, encased in a shell composed of a Ni-Cu alloy containing 75% copper. Nickels, made prior to 2019 are have the same composition as the cladding of dimes and quarters, but without the copper core, while only pennies have a pure copper cladding sandwiching a zinc core. It is well established that copper is an effective anti-viral agent mostly due to its ionization in aqueous solution and the formation of reactive oxygen species (ROS) (Grass et al., 2011; Rakowska et al., 2021; Vincent et al., 2018). In contrast, in cooper alloys, the anti-viral effectiveness is dependent on the copper content. Warnes et al. (2015b) have shown that at 70% the alloys is ineffective, while a reduction of more than 5 log was reported for 80% (Govind et al., 2021). Given the possible redistribution of components at the surface of coins that have been in circulation under unknown environmental conditions, the copper content of the coins is listed in Table 2. From the table, we can see that the pennies were indeed pure

copper without any zinc. On the other hand, the surfaces of the other coins contained mostly 72% copper, where the reduction relative to the stated values was due to the observation of various oxides. Hence us are results are consistent with those reported in reference (Govind et al., 2021; Warnes et al., 2015a, 2015b).

The infectivity of viral particles was also measured after drying at ambient temperatures on aluminum, polystyrene and polylactic acid. While no significant differences were observed between these materials, the data showed that they were comparable to the coinages with the copper alloy surfaces, where only a 2-log reduction occurred after 24 h. A 6-log reduction was observed after 72 h, defining the limit of infectivity from surfaces other than pure copper.

4.3. *Effectivity of HOCl fogging*

Disinfection of these surfaces is then critical, but pose some technical problems. First, the disinfectant while effective against viruses or bacteria, must not be toxic to humans and the environment. Second, these large public areas also have irregular surfaces where the virus can be deposited. An ideal solution therefore is HOCl, where HOCl has been shown to be minimally toxic to humans. Previous work using HOCl involved dipping or coating with the liquid form (Eryilmaz and Palabiyik, 2013; Wang et al., 2007), but fogging, which is known to be an effective method for disinfecting large irregular areas, had not been explored. In order to test the efficacy of the technique for H1N1 virus, we designed a special enclosed chamber which could be fogged with a controlled fog flow. Metal coupons were then placed in this chamber in both horizontal and vertical positions, and LRV was measured. The results confirmed that fogging was an effective method, where both continuous and pulsed fogging modes could achieve 6-log reduction. On the horizontal coupons this occurred after only one application for 15 s, since the fog condensed easily. On the vertical coupons, where the fog does not accumulate, this was achieved after two cycles of 15 s, 10 min apart or continuous fogging for 5 min.

The chlorine content was measured in sealed containers and found to decrease from approximately 600 ppm to 480 ppm after 9 months, and the pH was maintained around 5. In open containers, the pH was still maintained, but the chlorine content was reduced to approximately 200 ppm. Regardless, continuous fogging with low chlorine content solution, we were still able to achieve 6-log reduction, but with an extended time of 7.5 min. In sum, these results confirm that HOCl fogging can be a practical method for disinfection of public areas.

5. Conclusion

We studied the impact of common environmental factors on enveloped viruses and found that the virus was fairly resistant to extreme pH ranging from 4 to 11 and high saline concentrations up to 5 M, where the infectivity was reduced by less than one log. The largest factor affecting infectivity was temperature, where a 6-log reduction occurred after heating for only 10 min at 55°, while infectivity was maintained with only a one log reduction when samples of virus were stored between 4 °C or -20 °C for up to 6 days. Infectivity of the virus was reduced by 4 logs after 48 h when deposited on common surfaces such as polymers, aluminum, and common coinage with the exception of pennies, where nearly complete loss of infectivity (7 logs) occurred after 24 h. Intermittent fogging for only 5 min with HOCl produced a 6-log reduction at full strength of 550 ppm, and 7.5 min when the open containers were stored under ambient for 9 months where the chlorine content was reduced by 50%. These results indicate that HOCl fogging is very effective for disinfection of either polymer or metal surfaces.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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