Final Report

Envirolyte's Electrolysis Anolyte Water for Virus Inactivation study

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Abstract

The main purpose of this study is to estimate the efficacy of reducing virus activity by incubation viruses *in vitro* with Envirolyte's Electrolysis Anolyte Water. In the virus inactivation study, the enveloped viruses, herpes virus type 1 (HSV-1), influenza A and influenza B, as well as non-enveloped viruses, adenovirus and enterovirus, are selected for assays. Tissue Culture Infection Dose 50 (TCID50) and Plaque Assay (PA) methods are put in use as an index of virus inactivation for evaluation the effectivity of virus inhibitory rotio with Envirolyte's Electrolysis Anolyte Water treatment.
The inhibitory ratio of virus growth for all virus strains in assays reach the manifestly inhibitory criterion (99.9%) while the electrolyte water at original chloride concentration (410ppm) is in use. The deactivated efficacy for the electrolyte water treated to different virus strain is significant with various dilution factors while chloride concentrations are descented (i.e., 41ppm or 4.1ppm). Consequently, the inhibitory ratio from high to low rank as follows: Influenza A/H3N2, Echo6, 2009 pandemic influenza A/H1N1, seasonal Influenza A/H1N1, Enterovirus type 71, Influenza B, HSV-1, Coxsackie B5, Adenovirus.

Purpose

In recent years, emerging infectious diseases or re-emerging infectious diseases by viruses appear frequently, even sometimes to large scale outbreak, with large extent of human activities, climate changes, or ecological changes. In spite of outbreaks, hospital-acquired infection is quite common and direct trammission is a main pathway for such infections. Deactivating virus in virus opportunistic environment is an optimal means to intercept virus infection.

The disinfestant in common use is liquid bleach, i.e., sodium hypochlotire solution, but chlorination of drinking water can oxidize organic contaminants, producing trihalomethanes (also called haloforms), which are carcinogenic. For salfty reason, one reliable disinfectant with features of being friendly to living beings and ecological environment and of effects in reducing pathogenic activity is demanded.

Rencently, technologic industry develops a technique, anolyte elcetrolysis with multiple ceramic membranes as separatory functions, to produce neutral pH disinfectant solution, HClO including agent, as to overcome the defects of liquid bleach.

The purpose of this study is to estimate the efficacy of virus inactivation with the HClO including disinfectant agent incubation.

Materials and methods

The test material, Envirolyte's Electrolysis Anolyte Water, is obtained from TAIWAN I-SO BIOTEC CO., LTD.

The methods of this study are listed as follows,

1. Host cell cultures
   Obligated cell lines from liquid nitrogen stocks are prepared. After defrosting and ultracentrifugation, lift pellets out and plant them into 1% BSA medium culture plates for activating cells and then transfer cells to culture bottles. Incubate culture bottles at 5% CO2 and 37°C and subculture for each 3-4 days until cells form a single layer. Pour out culture medium from bottle and rinse with PBS buffer twice and then incubate with trypsin for 3-10 minutes at 37°C to strip away surface-attached cells, re-plated and incubated for 10 additional hours to prepare for further virus infection.

2. Virus cultures
   Seed 100~200 µl virus supernatant to host cell tubes with incubation for 1-2 hours at 5% CO2 and 37°C for infecting host cells and then observe cytopathic effect, CPE, then record for analysis. H1N1 (2009 panademic influenza A/H1N1), H1N1 (seasonal influenza A/H3N2,A/H1N1), and Influenza B cultured with MDCK cells. Echo virus, Coxsackie B, and enterovirus type 71 clutured with RD cells. Adenovirus and herpes simplex virus type 1 cultured with Hep-2 cells.
3. Virus Plaque Assays
   Seed virus tests of 10X serial dilution for virus supernatant with cultural medium to 6-well host cell dish (diameter 35 mm), at condition of each 2 wells for the same virus concentration and incubate at 37°C for virus infection. After 1 hour, suck medium out and pour 0.5% agarose in the dish and incubate dish at 5% CO2 and 37°C for 3 days. After 3 days, pour medium out and add formalin to fix cells and dye with crystal violet.
4. TCID50 titter test of virus infection
   Seed virus tests of 10X serial dilution for virus supernatant with cultural medium to 96-well host cell dish with condition of each 4 wells as replicated with the same 50 µl virus sample and 100 µl cultural medium as well as 150 µl cultural medium as control and incubate at 5% CO2 and 37°C for 72 hours for virus infection. Analysis with Reed-Meunch calculation tittle method for the tests.
5. Virus inactivity assay
   Test system: mix 0.1 ml virus stock with 0.9 ml Envirolyte's Electrolysis Anolyte Water by vortex mixer and stand at room temperature for 10 min.
   Control system: mix 0.1ml virus with 0.9 ml PBS by vortex mixer and stand at room temperature for 10 min.

6. Materials
   Host cell lines: MDCK, RD, A549,Vero

   Virus strains: Influenza A H1N1 (H090135)

   Influenza A H3N2 (H090103)

   Influenza A swine H1N1 (VI110809)

   Influenza B (B110439)

   Herpes simplex virus type 1 (D110647)

   Enterovirus type 71 (VI061482)

   Coxsackie B5 (D100302)

   Echovirus type 6 (D110604)

   Adenovirus (H3457)

   Medium: MEM, DMEM, FBS,TPCK-trypsin, E-2, E-10, D-10, E0-TPCK trypsin

Concentration of chloride content in Envirolyte's Electrolysis Anolyte Water: 410ppm in mean.

Results

1.Inactivation
Mix 0.1 ml virus stock with 0.9 ml Envirolyte's Electrolysis Anolyte Water by vortex mixer and stand at room temperature for 10 min for quantitative estimation.

Plaque assays for Enveloped viruses (Influenza A H1N1, Influenza A H3N2, Influenza A swine H1N1, Influenza B, Herpes simplex virus type 1).

TCID50 assay for Non-enveloped virus (Enterovirus type 71, Coxsackie B5, Echovirus type 6, Adenovirus).

	Virus Control	Test	Inactivation ratio(%)
Influenza A/H1N1 (seasonal)	8.35×10^{6}/mL	<100/mL	99.9988
Influenza A (H3N2)	4.8×10^{5}/mL	<100/mL	99.979
2009 pandemic influenza A/H1N1	2.7×10^{5}/mL	<100/mL	99.963
Influenza B	1.3×10^{6}/mL	<100/mL	99.992
HSV-1	1.155×10^{7}/mL	<100/mL	99.999
Enterovirus 71	$TCID_{50}=10^{-4.6}$	$TCID_{50}<10^{-1}$	99.98
Coxsackie virus B5	$TCID_{50}>10^{-7}$	$TCID_{50}<10^{-1}$	99.9999
Echovirus 6	$TCID_{50}=10^{-5.88}$	$TCID_{50}<10^{-1}$	99.987
Adenovirus	$TCID_{50}=10^{-5.4}$	$TCID_{50}<10^{-1}$	99.996

Summary: The inhibitory growth ratio at the concentration of chloride content, 410ppm, reach the criterion of 99.9% with viruses incubation in Envirolyte's Electrolysis Anolyte Water for 10 minutes.

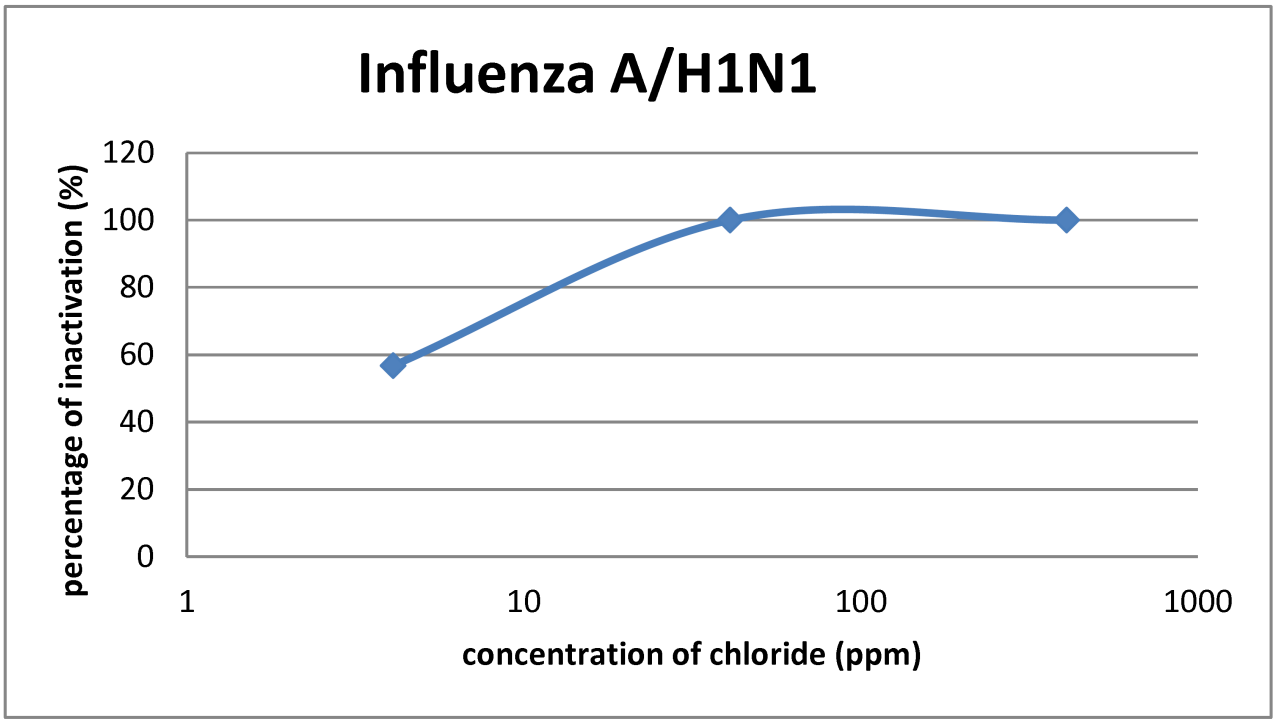
2.Endpoint test
Dilute 1X
Envirolyte's Electrolysis Anolyte Water with PBS at factor of 10X and 100X, respectively and mix 0.1 ml virus stock with each stock of 0.9 ml Envirolyte's Electrolysis Anolyte Water by vortex mixer and stand at room temperature for 10 min for quantitative estimation.

Plaque assay for Enveloped virus (Influenza A H1N1, Influenza A H3N2, Influenza A swine H1N1, Influenza B, Herpes simplex virus type 1).

TCID50 assay for Non-enveloped virus (Enterovirus type 71, Coxsackie B5, Echovirus type 6, Adenovirus).

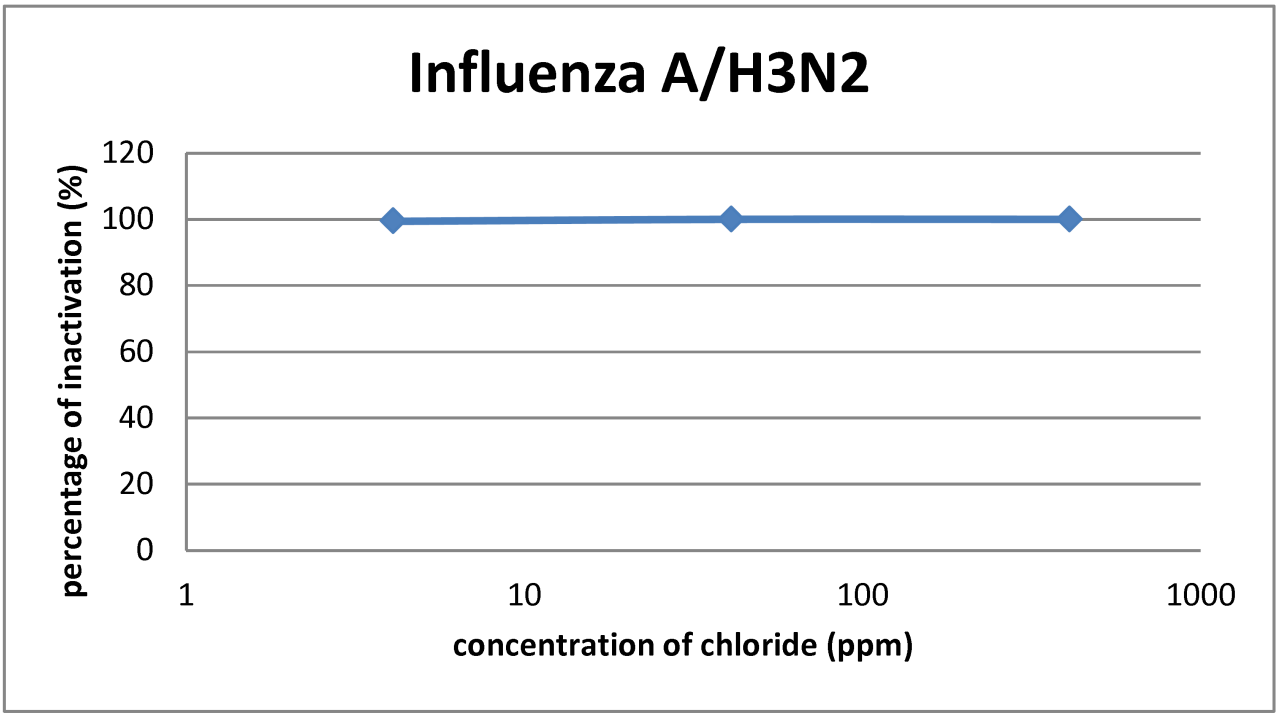
(A) Influenza A/H1N1

	Titers (pfu/ml)	Percentage of inactivation(%)
PBS(control)	1.85×10^{7}	
410 ppm	$<10^{2}$	99.9995
41 ppm	3×10^{2}	99.9983
4.1 ppm	8×10^{6}	56.757



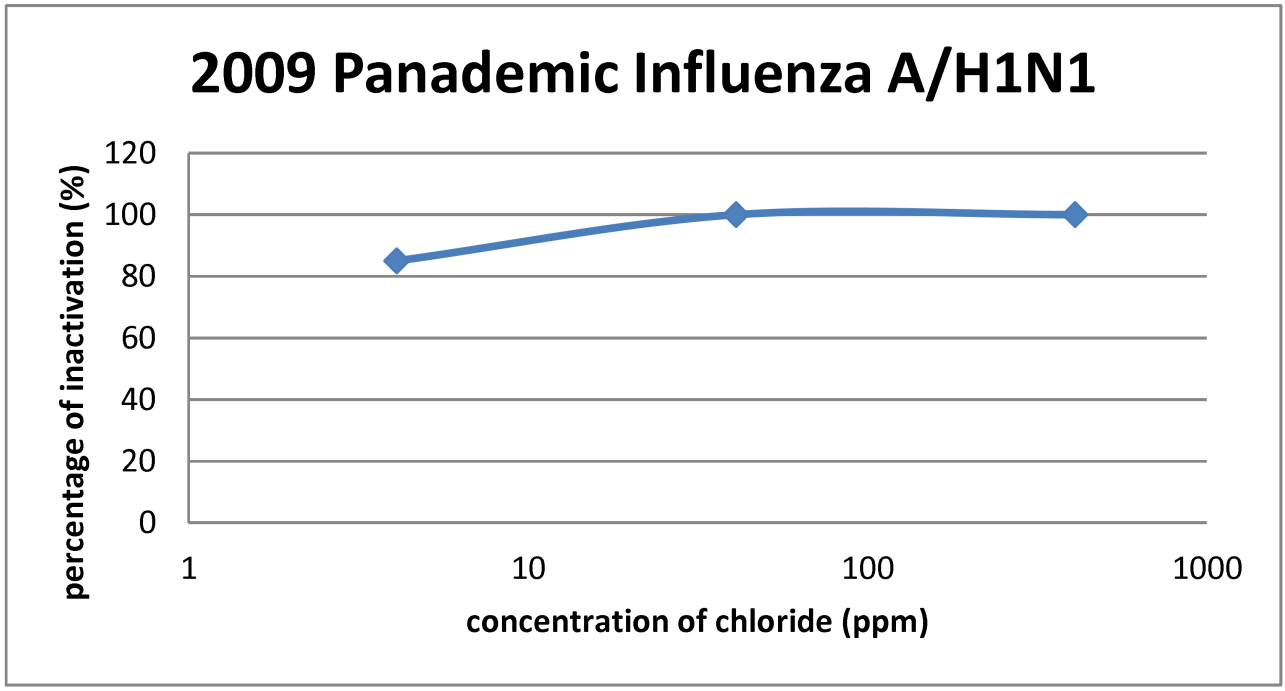
(B) Influenza A/H3N2

	Titers (pfu/ml)	Percentage of inactivation(%)
PBS(control)	9.5×10^{6}	
410 ppm	$<10^{2}$	99.999
41 ppm	$<10^{2}$	99.999
4.1 ppm	5.65×10^{4}	99.405



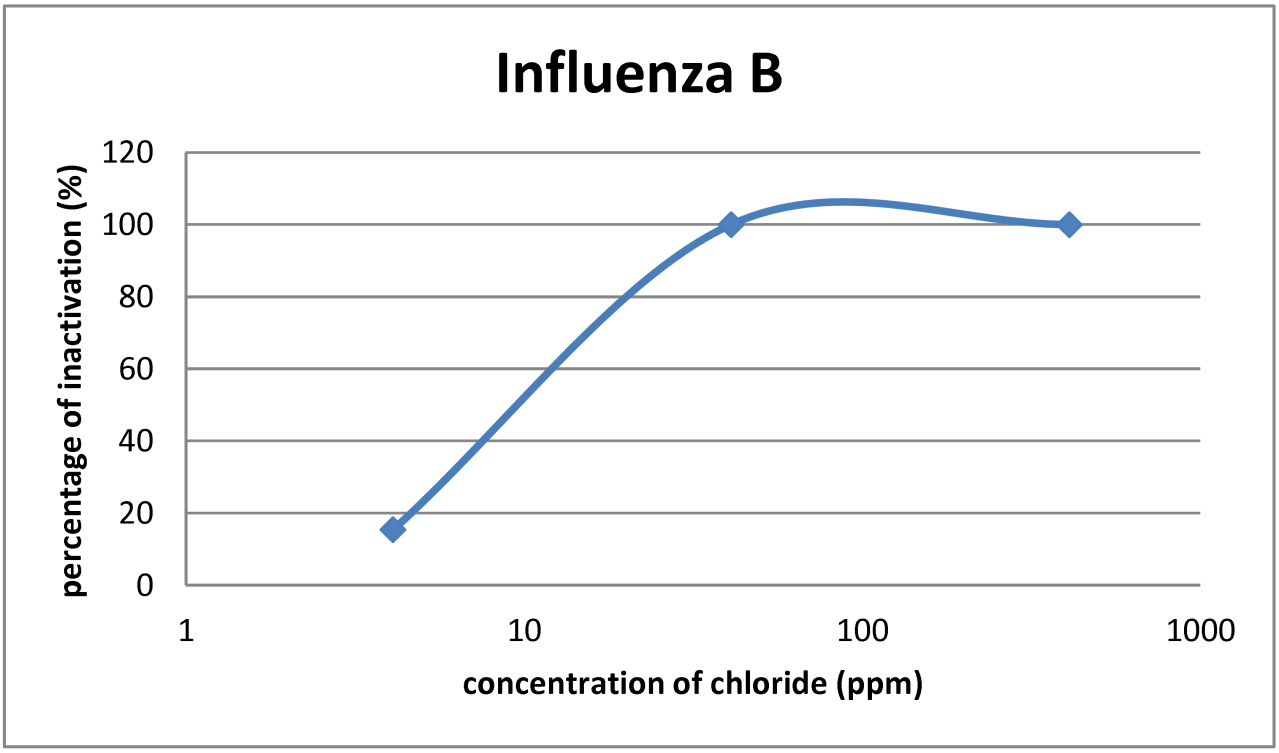
(C) 2009 Panademic Influenza A/H1N1

	Titers (pfu/ml)	Percentage of inactivation(%)
PBS(control)	9×10^5	
410 ppm	$<10^2$	99.989
41 ppm	$<10^2$	99.989
4.1 ppm	1.35×10^5	85



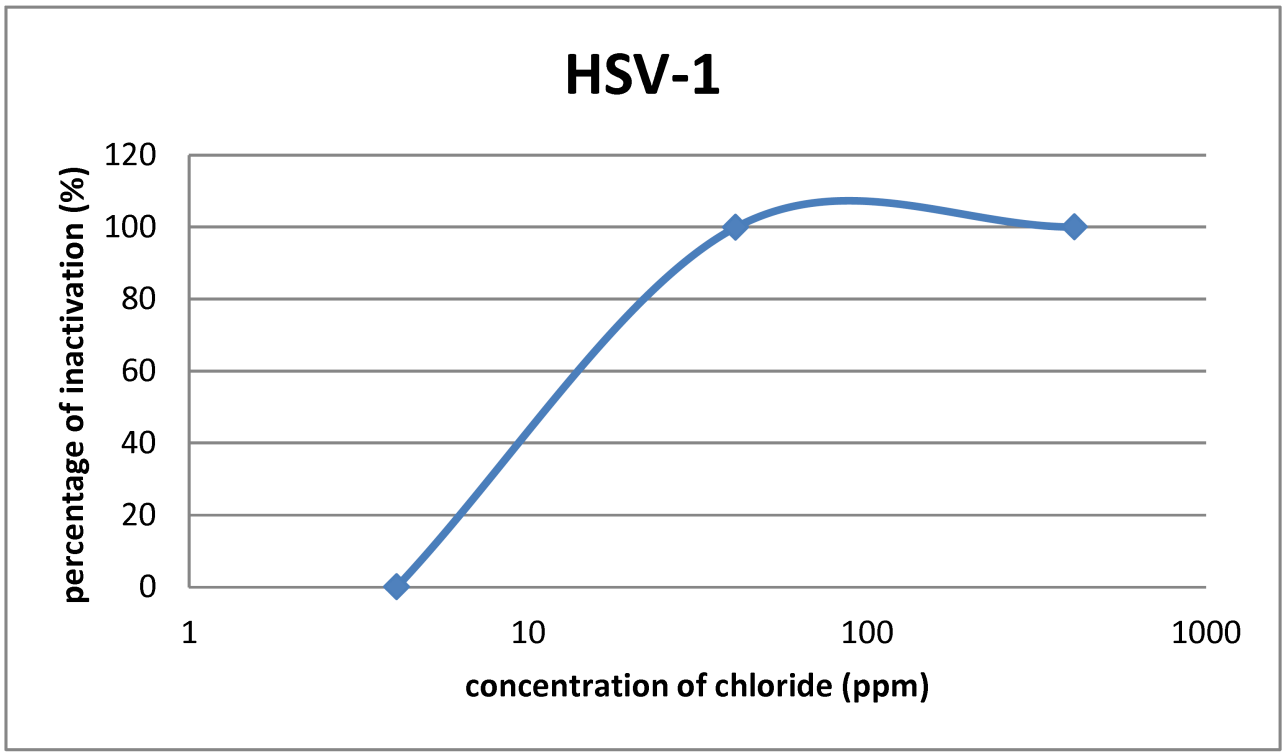
(D) Influenza B

	Titers (pfu/ml)	Percentage of inactivation(%)
PBS(control)	1.3×10^{6}	
410 ppm	$<10^{2}$	99.992
41 ppm	$<10^{2}$	99.992
4.1 ppm	1.1×10^{6}	15.4



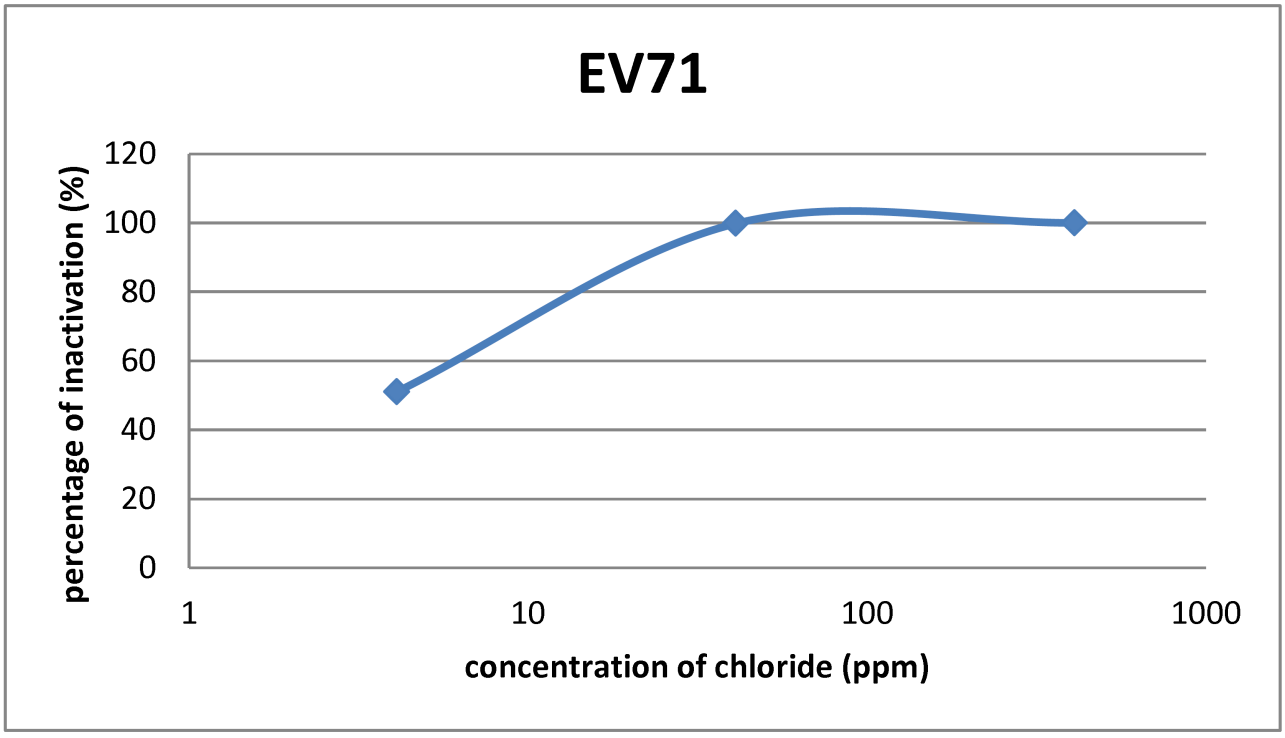
(E) Herpes simplex virus type 1

	Titers (pfu/ml)	Percentage of inactivation(%)
PBS(control)	1.155×10^{7}	
410 ppm	$<10^{2}$	99.999
41 ppm	1.54×10^{4}	99.867
4.1 ppm	1.185×10^{7}	Non-effective



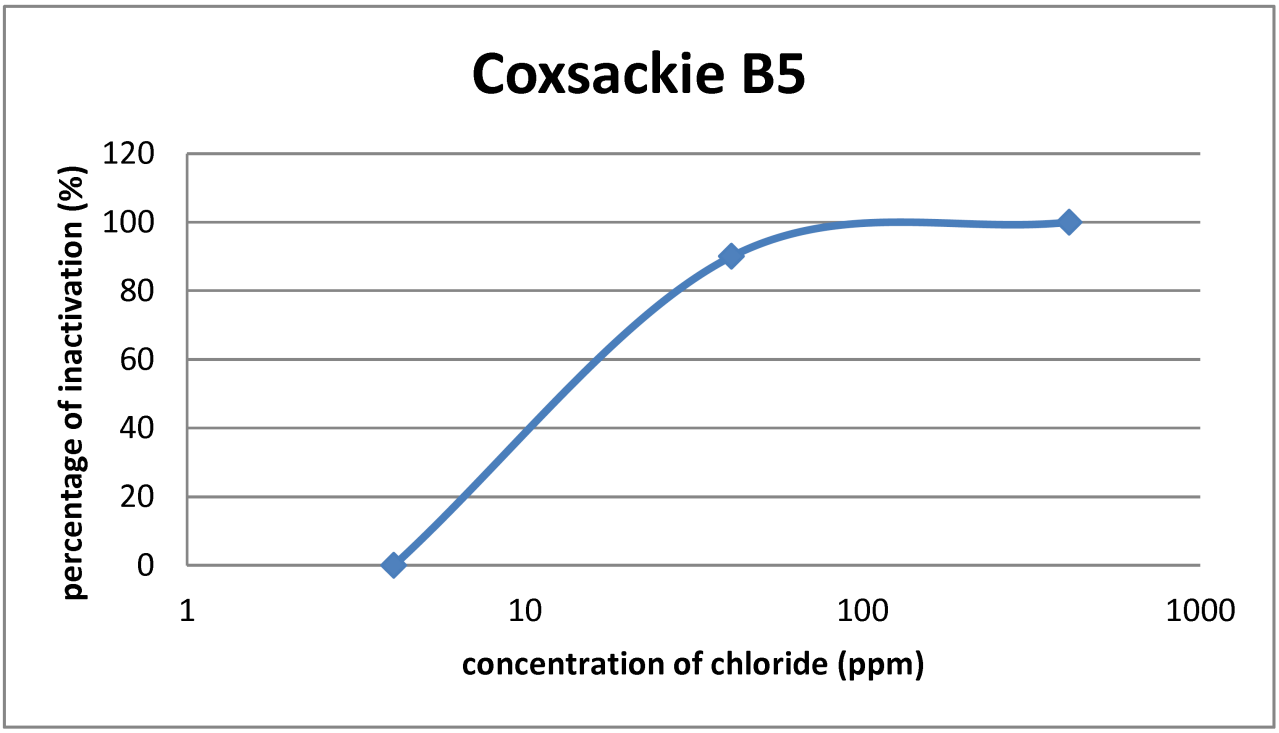
(F) Enterovirus type 71

	Test	Percentage of inactivation(%)
PBS(control)	$TCID_{50}=10^{-6.31}$	
410 ppm	$TCID_{50}<10^{-1}$	99.9995
41 ppm	$TCID_{50}=10^{-3.64}$	99.79
4.1 ppm	$TCID_{50}=10^{-6}$	51.02



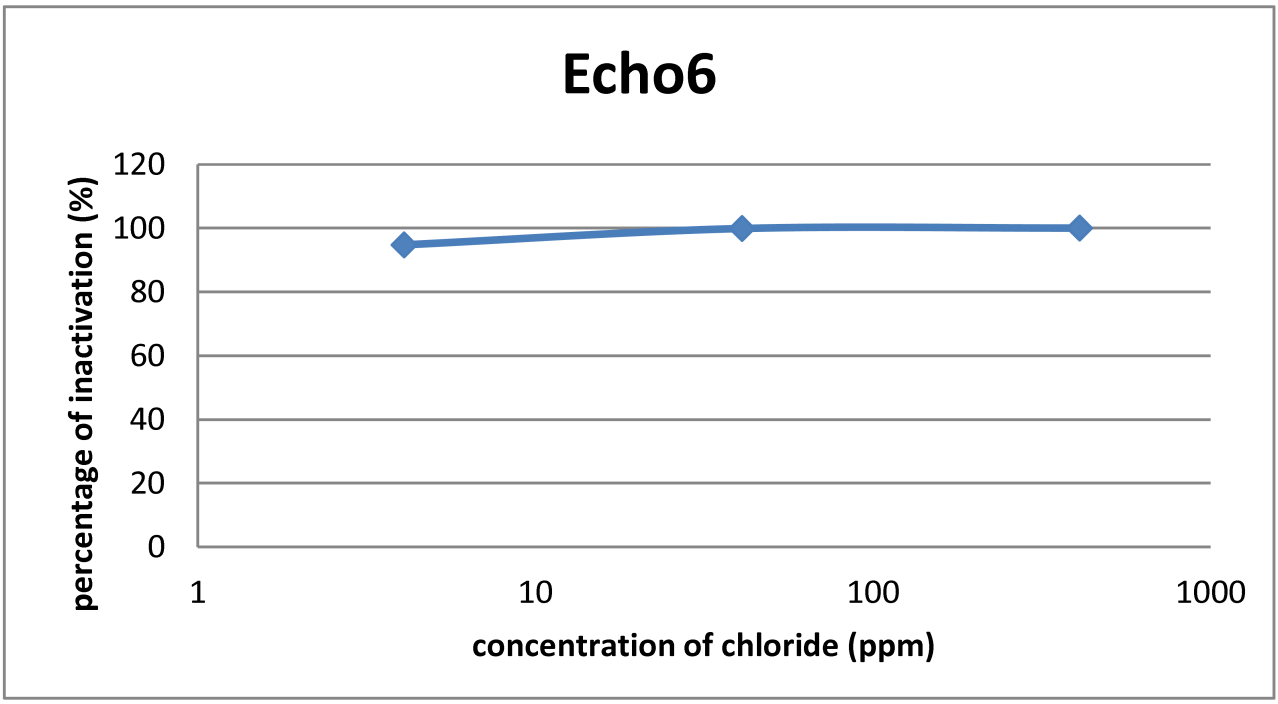
(G) Coxsackie B5

	Test	Percentage of inactivation(%)
PBS(control)	$TCID_{50}>10^{-7}$	
410 ppm	$TCID_{50}<10^{-1}$	99.9999
41 ppm	$TCID_{50}=10^{-6}$	>90
4.1 ppm	$TCID_{50}>10^{-7}$	Non-effective



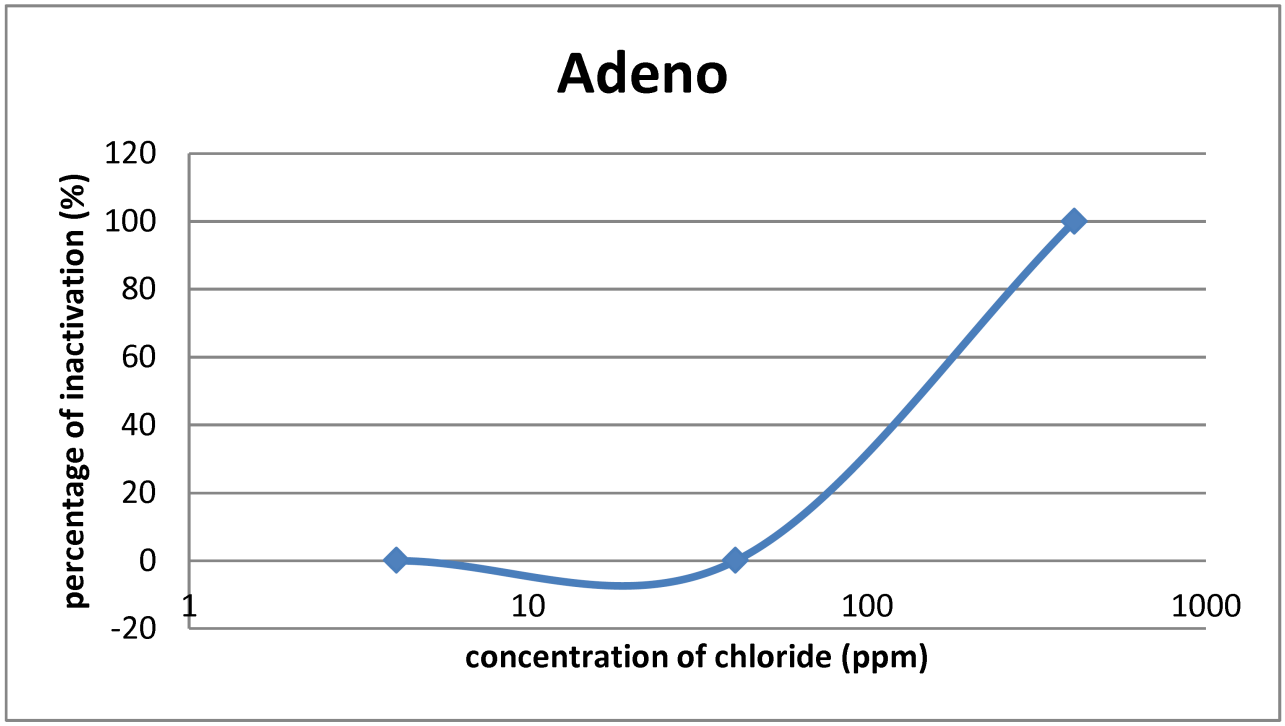
(H) Echovirus type 6

	Test	Percentage of inactivation(%)
PBS(control)	$TCID_{50}=10^{-5.88}$	
410 ppm	$TCID_{50}<10^{-1}$	99.9987
41 ppm	$TCID_{50}=10^{-3}$	99.868
4.1 ppm	$TCID_{50}=10^{-4.6}$	94.752



(I) Adenovirus

	Test	Percentage of inactivation(%)
PBS(control)	$TCID_{50}=10^{-5.4}$	
410 ppm	$TCID_{50}<10^{-1}$	99.996
41 ppm	$TCID_{50}=10^{-5.5}$	Non-effective
4.1 ppm	$TCID_{50}=10^{-5.4}$	Non-effective



Summary

At 410ppm chloride concentration, all test viruses under electrolyte water treatment for 10 minutes reach 99.9% criterion of inhibitory ratio. Exceptions of adenovirus and coxsackie virus, other test viruses still reach 99.9% criterion of inhibitory ratio at 41ppm chloride concentration. But inhibitory ratio varied much to test viruses while chloride concentration decreased to 4.1ppm.

Conclusion

All test viruses are sensitive to the electrolyte water at 410ppm chloride concentration. With dilutions of the electrolyte water, the effects of inhibitory ratio to different viruses are differential to each other. Further analysis regarding the survival curves to different viruses, RNA viruses are more sensitive to the electrolyte water than DNA viruses whether the envelope exhibits or not. Subtypes of the same virus, such as Influenza virus, are differently sensitive to the electrolyte water.