Infection Control: Relationship of Subtype Influenza A Pandemic Strains to Virucidal Activity of a Quaternary Ammonium Disinfectant

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INTRODUCTION: Spread of influenza infection is mitigated not only through antiviral agents and vaccination but also by treatment of environmental surfaces with surface disruptive chemical germicides. Little data is available on the comparative susceptibility of pandemic strains of Influenza A to chemical agents. We have embarked on a systematic study of the effect of various germicides on strains of influenza. The present work deals with Benzalkonium chloride, a dual active antimicrobial agent accepted world-wide as both a disinfectant and antiseptic and various subtypes of Influenza A.

HISTORY: The major antigenic changes in the influenza genome over the past fifty years have involved hemagglutinins H1, H2 and H3 producing the pandemics of 1957 (A/Japan 305/57/H2N2), 1968 (A/Hong Kong/8/68/H3N2) and the novel swine flu pandemic of 2009. (A/Cal/2009/H1N1) These are the subtypes that we have studied. Clearly, the question arises as to whether the changes in antigencity are coupled with changes in germicide susceptibility.

METHOD: We have employed both quantal and plaque methods (ASTM E-1052) in a MDCK cell culture system employing serum-free medium supplemented with trypsin. Microscopic examination of cytopathic effect (CPE) was the marker for infectivity.

RESULTS: The log inactivation and percent inactivation after a 60 second contact time for the H1, H2 and H3 pandemic strains are shown to the right ranging from 99.97%-99.999%.The data shows that the three hemagglutinin subtypes were highly susceptible to the Quaternary Ammonium Compound in the short term *in vitro* experiments.

CONCLUSION: The appearance of novel subtypes in the future can be met with the assurance that disinfectant and/or antiseptic resistance will be unlikely. Certainly, from the above data, although genetic reassortment of human and swine viruses may modulate influenza pathogenesis and limit existing vaccine benefit, it is not likely be a factor in control of viruses on environmental surfaces by Benzalkonium-type disinfectant/cleaning agents in community or health care environments.

High Titer Viral Pools were Used to Perform these Experiments

Antiviral = Benzalkonium chloride 60 Second Contact Time		H1N1 A/Swine/ Iowa/15/30				H1N1 A/Swine/ Cal/04/09				H2N2 A2/J305				H3N2 A2/Hong Kong/8/68				Representative Inactivity All subtypes			
Viral Infectivity Assay	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	T	T	T	T
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	T	T	T	T
	10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-4}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-5}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-6}	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-7}	0	0	0	0	+	+	+	0	0	0	0	0	+	+	+	+	0	0	0	0
	Cell control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$TCID_{50}$		$10^{6.0}$				$10^{7.3}$				$10^{6.5}$				$10^{7.5}$				$\leq 10^{2.5}$			
Δ Log		**≥ 3.5**				**≥ 4.8**				**≥ 4.0**				**≥ 5.0**				**Legend: 0 = No Virus; T = Cytotoxic; + = Virus**			

Plaque Assay with H3N2 A2/Hong Kong/8/68

Viral potency is also determined by counting the number of plaques with the naked eye, marking each plaque then confirming with microscopy.

Left Panel: In this study, there was excessive neutral red precipitation in agarose overlay during incubation and therefore the agarose overlay was removed. Crystal violet staining was carried out on the same plate. Some parts of cell monolayer peeled off in the process of removing agarose overlay. The titer based on reading by naked eye and microscopy: 1.3 x 10^6 pfu/mL (41 plaques in the well inoculated 3 mL of 10^{-5} virus dilution)

Right Panel: In this experiment staining was performed with Neutral Red. About 100 plaques are visible.

These experiments are intended for the sponsor to substantiate to US FDA that their antiviral substance is stable and effective.