University of New South Wales (UNSW)
Microbiology testing Laboratory
School of Optometry and Vision Science (SOVS)
Faculty of Medicine and Health
Sydney, NSW 2052, Australia

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ANTI-VIRAL EFFICACY OF SAN-AIR PRODUCTS AGAINST H1N1 INFLUENZA A VIRUS

1. TESTING OF H1N1 INFLUENZA A VIRUS AGAINST SAN-AIR GEL WITH ZINC AND HOUSEHOLD GRADE DISINFECTANT LIQUID

AIM:

To evaluate the antiviral efficacy of San-Air gel with zinc (SAGZ) and San-Air household disinfectant (SAHD) liquid against H1N1 influenza A virus

MATERIALS:

1. H1N1 influenza A virus (A/PR/8/34 ATCC VR-1469)
2. MDCK cells (ATCC: MDCK.2 CRL-2936)
3. San Air gel with zinc
4. San Air Household Grade Disinfectant
5. Cell culture medium (Dulbecco's Modified Eagle Medium; DMEM)
7. 24-well tissue culture plates
8. Trypsin TPCK (Sigma, USA)
9. 10% w/v bovine serum albumin
10. 1% penicillin-streptomycin
11. 0.5% w/v crystal violet stain
12. Centrifuge tube

METHODS:

The Tissue Culture Infectious Dose 50 (TCID50) assay was used to assess the antiviral effects of MDCK cells were then seeded at a density of 0.5 x 10^5 cells per well in a 24-well plate and cultured overnight at 37 °C in 5% CO_2. SAGZ and SAHD liquid against the H1N1 influenza A

virus. Initially, SAGZ and SAHD were weighed (5 grams). SAGZ was then incubated in a sonicator at 45°C for 10 minutes to convert it from a gel to a liquid. For SAGZ, a 1/10 and 1/100 dilution series was prepared using plain DMEM solution, and added to a final viral inoculum of 1.0 x 10^5 Tissue Culture Infectious Dose 50 ($TCID_{50}$)/mL. For SAHD liquid, dilutions of 1/10, 1/32, 1/64 and 1/100 were prepared using plain DMEM solution, and added to a final viral inoculum of 1.0 x 10^5 $TCID_{50}$/mL. All aliquots were incubated at 37°C for one hour. For the control, a similar virus concentration was incubated in DMEM media alone for the same duration as the test. After incubation, ten-fold serial dilutions of each test were performed, and 100 µL from each dilution was added to four wells containing the MDCK cells. Cytopathic effects (CPE) were observed four days post-infection. The dilution at which 50% of the well's exhibited infection was determined using the Reed and Muench method. The $TCID_{50}$/mL of the test and control were calculated and compared to draw conclusions regarding the antiviral activity of SAGZ and SAHD liquid.

RESULTS:

There was 2182 viruses in the control (Table 1). Incubating the virus with SAGZ for an hour reduced the number of viable viruses by 99% for 1/10 dilution and 90% for 1/100 dilution compared to the untreated controls for SAGZ. For SAHD, the number of viable viruses was reduced by 100% for 1/10 dilution, 99% for 1/32 dilution, 98.5% for 1/64 dilution, and 90% for 1/100 dilution.

Table 1: Ability of SAGZ and SAHD liquid to reduce the number of viable H1N1 influenza A virus particles.

Dilution	**1/10**	**1/32**	**1/64**	**1/100**
Control ($TCID_{50}$/mL (number of viable virus)	2182			
SAGZ $TCID_{50}$/mL (number of viable virus)	22	-	-	218
SAHD $TCID_{50}$/mL (number of viable virus)	0	22	32	218

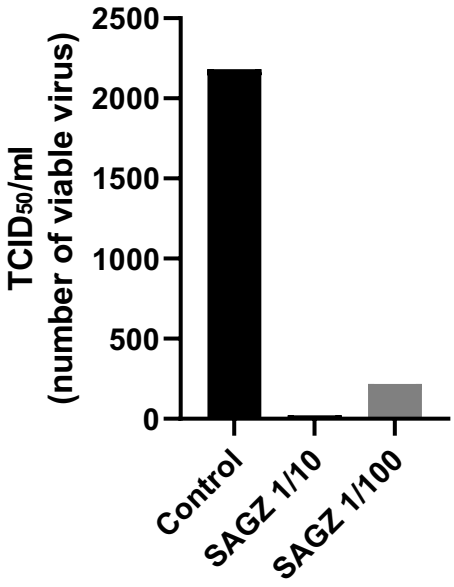

Figure 1: Number of viable H1N1 influenza A virus in $TCID_{50}$/ml for control and SAGZ

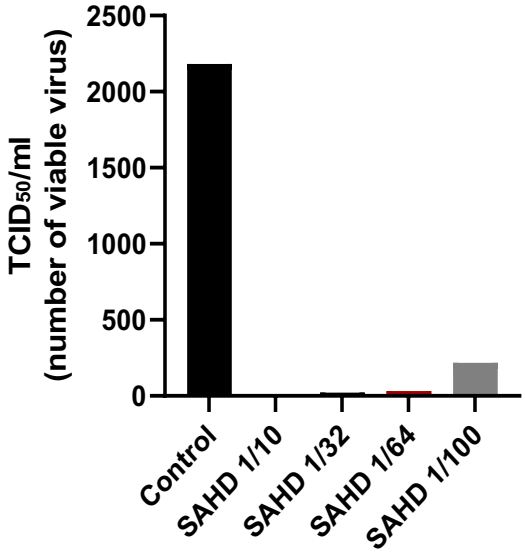

Figure 2: Number of viable H1N1 influenza A virus in TCID50/ml for control and SAHD

CONCLUSIONS:

SAGZ and SAHD liquid can significantly reduce the number of H1N1 influenza A virus that can infect cells.

2. AEROSOLS TESTING OF H1N1 INFLUENZA A VIRUS AGAINST SAN-AIR GEL WITH ZINC

AIM: To evaluate the antiviral efficacy of evaporated San-Air gel with zinc and San-Air liquid against H1N1 influenza A virus

MATERIALS:

1. H1N1 influenza A virus (A/PR/8/34 ATCC VR-1469)
2. MDCK cells (ATCC: MDCK.2 CRL-2936)
3. San Air gel with zinc
4. The Bacterial Filter Efficiency (BFE) Test Rig
5. Cell culture medium (Dulbecco's Modified Eagle Medium; DMEM)
6. 24-well tissue culture plates
7. Trypsin TPCK (Sigma, USA)
8. 10% w/v bovine serum albumin
9. 1% penicillin-streptomycin
10. 0.5% w/v crystal violet stain

METHODS:

The Bacterial Filter Efficiency (BFE) Test Rig (CH Technologies, USA) was used to produce viral aerosols (Figure 1).

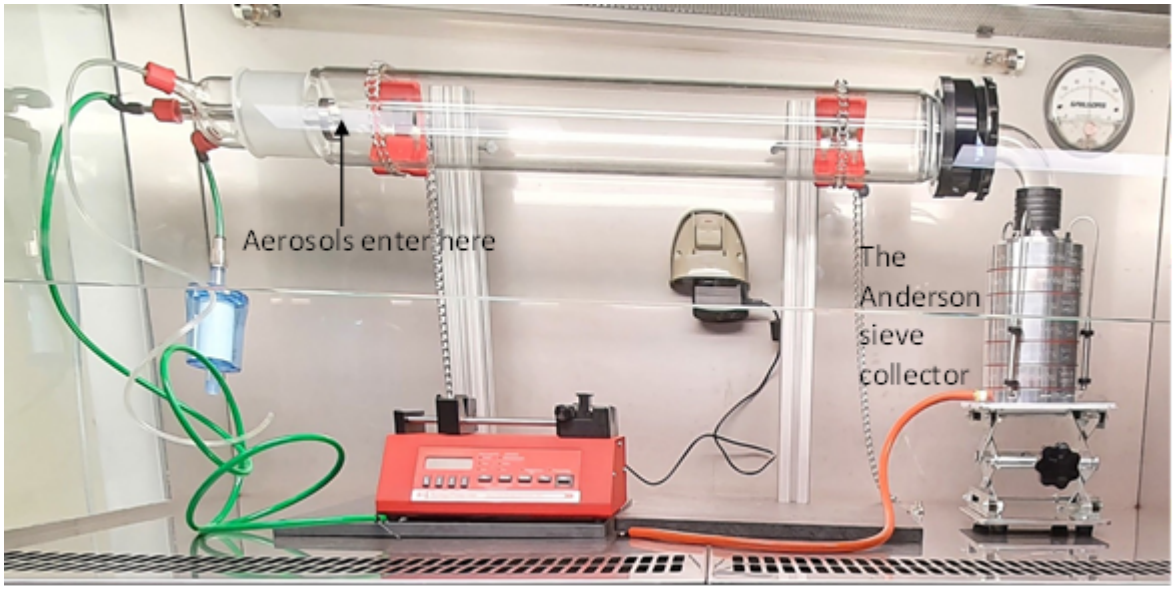

Figure 1: The Bacterial Filter Efficiency and Anderson sieve sampler.

The gel (10 g in total) was removed from its container and allowed to vaporise into the glass aerosol chamber (60 x 8 cm; 3016 cm^3) for 20 min prior to the introduction of the virus.The BFE Test Rig consists of a glass aerosol chamber, a 4-jet Blaustein Atomizer (BLAM) nebulizer nozzle, syringe pump, 6-stage viable cascade impactor (Anderson sieve collector; Figure 1) and a rotary vane vacuum pump. The viral inoculum (approximately 1.0 x 10^6 pfu/ml) was aerosolized using a continuous drive syringe pump set to deliver 50 µL of the inoculum for one minute through a nebulizer with an airflow of 28.3 L min-1. The nebulizer produces a mean particle size of 3.0 ± 0.3 µm which travels through the glass aerosol chamber and into the Anderson sieve sampler which contains agar plates to collect viable viruses. The 6-stage Anderson sampler uses perforated plates with progressively smaller holes at each stage, allowing particles to be separated according to size; the particle cut-off size for the first stage is 7 µm and 0.65 µm for the last stage. The air-flow was cut-off after 1 minute to stop aerosolization, however, the vacuum pump was allowed to run for a further 1 minute to collect residual aerosols from the glass chamber.

After 2 minutes, the agar plates were removed, 1.2 ml of 2 µg/mL Trypsin TPCK solution was added and the agar surface was scrapped using a sterile scrapper to collect the viruses. The antiviral activity of SAGZ against the H1N1 virus was evaluated using the Tissue Culture Infectious Dose 50 ($TCID_{50}$) assay. MDCK cells were seeded at a density of 0.5 x 10^5 cells per well in a 24-well plate and cultured overnight at 37 °C in 5% CO2. The virus aliquots from agar plates were serially diluted ten-fold for each test performed, and 100 µL from each dilution was added to four wells. Cytopathic effects (CPE) were observed four days post-infection. The dilution at which 50% of the well's exhibited infection was determined using the Reed and Muench method. The $TCID_{50}$/mL of the test and control were calculated.

Control runs were performed at the beginning of each experiment prior to the addition of 10.02 grams of the SAGZ placed in the glass aerosol chamber. The test run was conducted after the gel was allowed to evaporate into the glass aerosol chamber at ambient temperature for 20 minutes prior to exposure to the viral aerosols. The number viruses from each of the plates 3,4 and 5 per run was enumerated and added to calculate the total viral numbers.

RESULTS:

There was 502 virus in the control plates (Table 2). Leaving the SAGZ into the glass chamber for 20 minutes reduced the number of viable viruses in the aerosol by 37% compared to the untreated controls.

Table 2: Ability of vapourised San Air gel with zinc to reduce the number of aerosolised viable H1N1 influenza A virus particles.

Sample	Amount of SAGZ used	Exposure time (minutes)	$TCID_{50}$/mL (number of viable virus)	Log_{10} reduction	% Reduction
Control	-	-	502	-	-
San Air gel with zinc	10.02 g	20	317	0.2	37

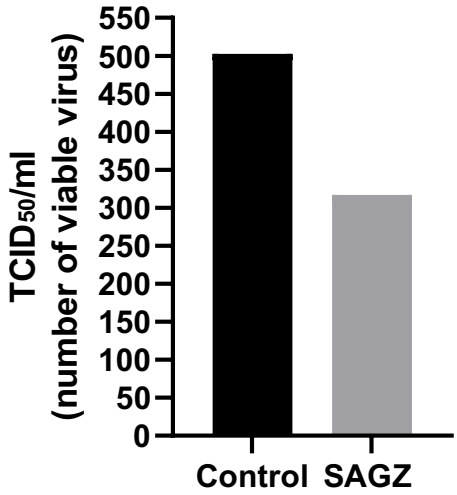

Figure 2: Number of viable H1N1 influenza A virus in PFU/ml for control and SAGZ

CONCLUSIONS:

Aerosols of SAGZ can significantly reduce the number of H1N1 influenza A virus that can infect cells.

Prepared by:

Dr Parthasarathi Kalaiselvan

Prof Mark Willcox

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