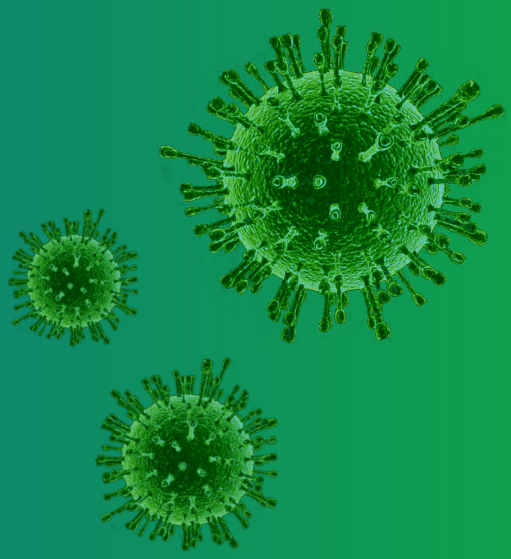
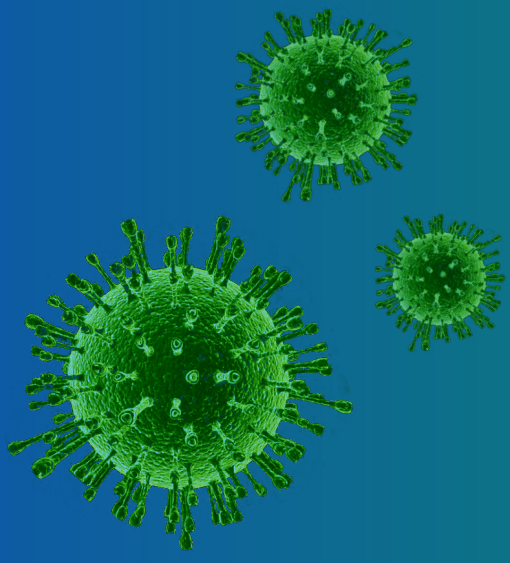


Nasaleze® & The Coronavirus

The Invisible Mask



A message from Paul Duxbury,
CEO of Nasaleze International

Introduction

2020 has indeed been a challenging year for all. We were busy putting our year plans into place in the 1st quarter with the focus on our Allergy products as usual but as the corona-crisis got underway we found ourselves with enormous demand on our Cold & Flu Blocker and Travel products.

The whole world went crazy for Vitamin C, Zinc, hand sanitisers, face masks and, well Nasaleze – The Invisible Mask.

Nasaleze Cold & Flu Blocker and Nasaleze Travel are marketed in the UK with the same formula. The differentiation initially was point of sale; we were trying to put the Travel product into airport pharmacies and keep Cold & Flu Blocker in the high street pharmacy.

As the UK went into lockdown and the high streets emptied it was the online sales that erupted – we had a 9000% increase online!

The bulk of demand in the UK at least went towards our Travel product. With people being concerned about leaving the house a Travel product offering protection against airborne germs & viruses in general, if not COVID-19, became a very attractive proposition.

At one point we were selling over 3,000 units per day on Amazon UK – 125 per hour on average. Amazon were in complete chaos and it became increasingly difficult to get the stock booked into their warehouse and made available for sale. We estimate sales would have been over the 5,000 unit per day level had the Amazon booking-in system kept pace.

As the virus went around the world the demand from our export markets followed it, with Russia in particular seeing a massive spike and being the 1st Nasaleze territory to order in excess of 1,000,000 bottles in single year (a feat achieved within 8 months actually, year to date).

We did not make any COVID-19 claims unlike some unscrupulous products – the demand was fuelled by the desire for prevention & protection – something Nasaleze has been promising since its birth in the early 2000's.

However, with our previous clinical trial data on rhinovirus and H5N1 virus we found ourselves the right product, at the right place at the right time.

As always we have very much been a company heavily reliant on proving ourselves so didn't lose much time in setting up an initial in vitro study to see if Nasaleze products could help with the crisis.

We found a suitable partner to fulfil the brief…
Perfectus Biomed. A company specialising in viral assays, including virucidal efficacy and viral barrier testing.

PERFECTUS BIOMED GROUP

The protocol was agreed, testing samples prepared and once restrictions had lifted sufficiently for us to get to the Post Office and received by Perfectus we were in business.

Over the next few pages you can see the outcome of the Perfectus study, written in conjunction with the Nasaleze Scientific Advisory Board.

In addition to the study there is also a press release from our PR Agency, Twelve.

Who with our own design team have created some suitable material to responsibly promote the benefits of Nasaleze for virus prevention, as a sensible add on to the WHO & various Government endorsed self-care strategy against COVID-19.

We wish to be clear and state we do not consider ourselves the silver bullet for COVID-19, but we do believe we can prove from the new and old data we have that our products are useful tools against viruses and hope we can play a part in helping people.

Best regards from all at Nasaleze.

Virucidal activity of Nasaleze® Cold & Flu Blocker and Nasaleze® Travel in cell cultures infected with human pathogenic coronavirus 229-E

Hunt N[1], Suleman L[1], Josling PD[2], Popov TA[3]

[1]Perfectus Biomed, Techspace One, Scitech Daresbury, Cheshire, UK
[2]Herbal Research Centre, Battle, UK
[3]University Hospital St. Ivan Rilski, Sofia, Bulgaria

Abstract

This *in vitro* study determined the anti-viral efficacy of a unique blend of powder cellulose supplemented with powdered garlic extract (PGE) and a signalling agent. The composition, presented as Nasaleze® Cold & Flu Blocker/Nasaleze® Travel, was assessed against Human Coronavirus 229E, CoV 229E {ATCC VR-740} in an *in vitro* experiment. The test substance was used at sub-optimal dosing levels to explore its prevention and treatment capabilities. The virucidal activity of this novel formulation was measured at 48, 72 and 112 hour periods after incubation. Results showed strong reductions in viral titre of Coronavirus 229E compared to a control, while no toxicity to human cells from the test formulation was noted. The extract Nasaleze® Cold/Travel showed potential to be used as a therapeutic and preventive agent.

The data reconfirms the established anti-viral activity of this formulation acting as a barrier preventing the virus from accessing the nasal mucosa and disrupting its replication.[1,2,3]

Introduction

The **COVID-19 epidemic in the United Kingdom** is part of the worldwide pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus reached the country in late January 2020. As of 30th August 2020, there have been 334,467 confirmed cases and 41,499 deaths of confirmed cases, the world's fourth-highest death rate. Worldwide more than 27 million cases and over 891,000 deaths have been recorded, with the United States, Brazil and India recording the highest number of cases. More than 90% of those dying had underlying illnesses or were over 60 years old.

In March 2020 , the UK government imposed an order, dubbed "Stay Home, Protect the NHS, Save Lives", banning all non-essential travel and contact with people outside one's home (including family and partners), and shutting almost all schools, businesses, venues, facilities, amenities and places of worship. Those with symptoms, and their households, were told to self-isolate 14 days, while those at higher risk due advanced age and accompanying comorbidities were told to shield themselves. People were told to keep apart in public. Police were empowered to enforce the measures, and the Coronavirus Act 2020 gave the government emergency powers not used since the Second World War.

The lengthy restrictions severely damaged the UK economy, lead to millions of job losses, worsened mental health and suicide rates, and caused "collateral" deaths due to isolation and decline of living standards.

In recent years, a number of countries in East and South-East Asia including China had seen an outbreak of various types of infectious flu including SARS Cov 1, MERS, H5N1 avian flu and now Coronavirus described as COVID-19. The infection mainly affected poultry (chickens and ducks) or bats, which were then wiped out in their hundreds of thousands.

The highly pathogenic avian flu virus arrived in Russia in July 2005 and to date the H5N1 flu virus has been recorded in many parts of the Russian Federation: in Western Siberia, in the Urals and in the Astrakhan province. This prompted the conduct of *in vitro* tests using Nasaleze® Cold/Travel which proved very successful at both destroying H5N1 and preventing its replication in human cell lines. This data was published in the European Journal for Nutraceutical Research[3]. Subsequently, a similar *in vitro* test against Coronavirus 229E which is part of the corona virus and common cold virus families with similar characteristics and structures were carried out. With the agent picked for this

evaluation we already had a history of success in controlling the viral agent H5N1, so our aim was to check if this Nasaleze® Cold/Travel formulation could be successful in both reducing viral load of Covid 229E and preventing its replication.

Material and methods

The test viral organism chosen was Human Coronavirus 229E and the utilised cell type was MRC-5. This Medical Research Council cell strain 5 is a diploid human cell culture line composed of fibroblasts, originally developed from lung tissue.

Cell maintenance and assay set-up

MRC-5 cells were used as the host cell line for human coronavirus 229E (CoV 229E) propagation. MRC-5 cells were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 20% Foetal Bovine Serum (FBS) and 1% penicillin-streptomycin (complete EMEM) at 37 ± 2 °C and 5% CO2. In preparation for the cytotoxicity screening and anti-viral assays, MRC-5 cells were seeded into 24 well plates at 1.0 x 105 cells/mL and incubated at 37 ± 2 °C and 5% CO2 for 24 hours, or until they reached 80-90% confluency. In preparation for tissue culture infectivity dose 50 (TCID50) testing, MRC-5 cells were seeded into 96 well plates at 2 x 105 cellsmL-1 and incubated at 37 ± 2 °C and 5% CO2 for 24 hours.

Phase 1: Checking for potential cytotoxic effects of the Nasaleze® Cold/Travel formulation **on the selected MRC-5 cell line**

Nasaleze® Cold/Travel was diluted to 3.2 mg/0.1mL, 6.4 mg/0.1mL and 12.8 mg/0.1mL in EMEM supplemented with 2% FBS and 1% penicillin-streptomycin (assay medium). Complete EMEM was aspirated from the test plates and 100 µL of each test concentration was added to duplicate wells. Following a 10-minute incubation period at 20 ± 2 °C an additional 400 µL of assay medium was added to the test wells. Plates were incubated for 24 hours at 37 ± 2 °C and 5% CO2. Following incubation, visual scoring was performed on a scale of 0 to 4 according to ISO 10993-5 guidelines (Table 1). Cytotoxic effects were assessed based on a variety of morphological changes to the MRC-5 cells such as cell rounding, detachment and cell lysis.

Visual Score	Cells with cytotoxic effects (%)	Reactivity classification
0	0	None
1	0 – 20	Slight
2	20 – 50	Mild
3	50 – 70	Moderate
4	70 – 100	Severe

Table 1. Cytotoxicity visual scoring and reactivity classifications.

Phase 2: Assessment of the preventative and virucidal capabilities of Nasaleze® Cold/Travel

MRC-5 cells were treated with Nasaleze® Cold/Travel according to two methods to determine the preventative and treatment capabilities of the formulation. The assays were performed in 24-well plates utilising duplicate wells for each experimental condition.

Preventive treatment of MRC-5 cells using Nasaleze® Cold/Travel before infection with high and low doses of human coronavirus 229E

To assess the preventative capabilities of Nasaleze® Cold/Travel against CoV 229E, MRC-5 cells were pre-treated with 3.2 mg of the formulation for 10 minutes before infection with CoV 229E multiplicity of infections (MOIs) of 1 (high dose) and 0.01 (low dose). Complete EMEM was aspirated from the test plates and washed once in Dulbecco's phosphate buffered saline (DPBS) before application of 3.2 mg Nasaleze® Cold/Travel in 100 µL assay media. Following a 10 minute incubation at 20 ± 2 °C, cells were inoculated with 100 µL CoV 229E, pre-diluted to achieve the high and low MOI infection, and incubated at 35 ± 2°C and 5% CO2 for 30 minutes. Infected cells were then supplemented with an additional 300 µL of assay medium and incubated at 35 ± 2 °C and 5% CO2 for four days. The cytopathic effect (CPE) of the virus on the MRC-5 cells was scored on days 2, 3 and 4 to the criteria described in Table 1. On days 3 and 4, 100 µL of media was harvested from each well to determine the viral titre before replacing with 100 µL of fresh assay medium. Harvested samples were stored at -80 °C until required for viral titre determination. It should be noted that 3.2mg of the test substance is sub optimal dosing and represents only 1 puff into only 1 nostril, whereas the product instructions indicate multiple dosing into BOTH nostrils to prevent or treat any type of airborne infection.

Treatment of human coronavirus 229E infected MRC-5 cells with Nasaleze® Cold/Travel

To assess the treatment capabilities of Nasaleze® Cold/Travel against CoV 229E, MRC-5 cells were first infected with high and low CoV 229E MOIs, 1 and 0.01 respectively, before treatment with the formulation. Complete EMEM was aspirated from the test plates and washed once in DPBS before being inoculated with 100 µL of pre-diluted CoV 229E to achieve high and low MOI infections and incubated at 35 ± 2 °C and 5 % CO2 for 30 minutes. Following incubation, viral inoculum was removed and a sub optimal 3.2 mg dose of Nasaleze® Cold/Travel in 100 µL assay media was added to the cells and incubated for 10 minutes at 20 ± 2 °C to allow the formation of the gel barrier. Treated cells were then supplemented with an additional 300 µL of assay medium and incubated at 35 ± 2 °C and 5% CO2 for four days. The CPE of the virus on the MRC-5 cells was scored on days 2, 3 and 4 to the criteria described

in Table 1. On days 3 and 4, 100 µL of media was harvested from each well to determine the viral titre before replacing with another 100 µL of fresh assay medium. Harvested samples were stored at -80 °C until required for viral titre determination.

Viral infectivity quantification by TCID50

To determine the viral titre of harvested samples, 10-fold serial dilutions were performed in assay medium. Medium was aspirated from the wells of the cell plate and cells were washed with DPBS. One hundred microlitres of each dilution of the samples were added to the corresponding test wells. Test plates were incubated at 35 ± 2 °C and 5% CO_2 for 7 days. There were four replicate wells for each test condition. After incubation, viral CPE was determined using an Olympus CK2 inverted microscope. The viral titre was calculated using the Spearman-Kärber method.

Results

Phase 1: MRC-5 cytotoxicity screen

There was no observable cytotoxicity in MRC-5 cells exposed to Nasaleze® Cold/Travel following a 24-hour contact time (Table 2). When visual scoring was performed, the gel barrier formed by Nasaleze® Cold/Travel was visible on top of the cell monolayer. Additionally, a residue was visible on treated cells (Appendix I).

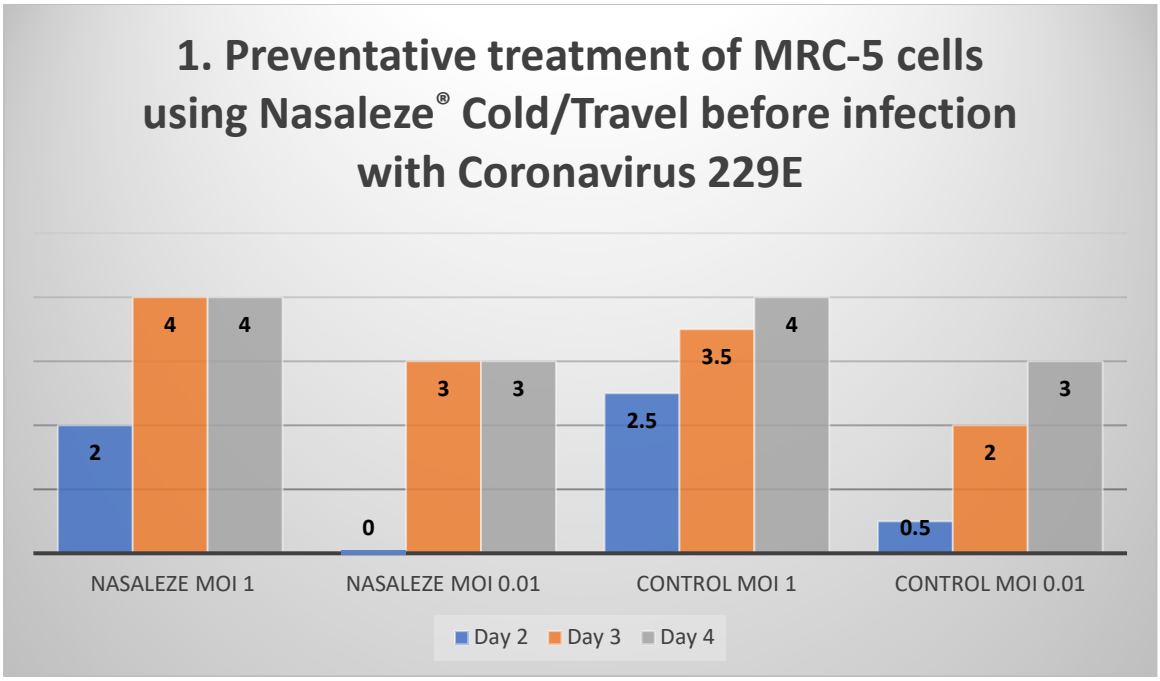
Treatment	Visual score	Reactivity classification
Nasaleze® Cold/Travel	0	No cytotoxicity

Table 2. Cytotoxicity of Nasaleze® Cold/Travel using visual scoring.

Preventive treatment of MRC-5 cells using Nasaleze® Cold/Travel before infection with coronavirus 229E

Cytopathic effect of CoV 229E on MRC-5 cells pre-treated with Nasaleze® Cold/Travel

Following a 2, 3 and 4 day or 48, 72 and 112 hours incubation period, the CPE of the test plate was scored (Chart 1). Representative images of the CPE observed are presented in Appendix II. Duplicate cells treated with Nasaleze® Cold/Travel with a high MOI of CoV 229E showed slight CPE on day 2 and severe CPE on days 3 and 4. Duplicate cells treated with Nasaleze® Cold/Travel with a low MOI of CoV 229E showed no CPE on day 2 and moderate CPE on days 3 and 4

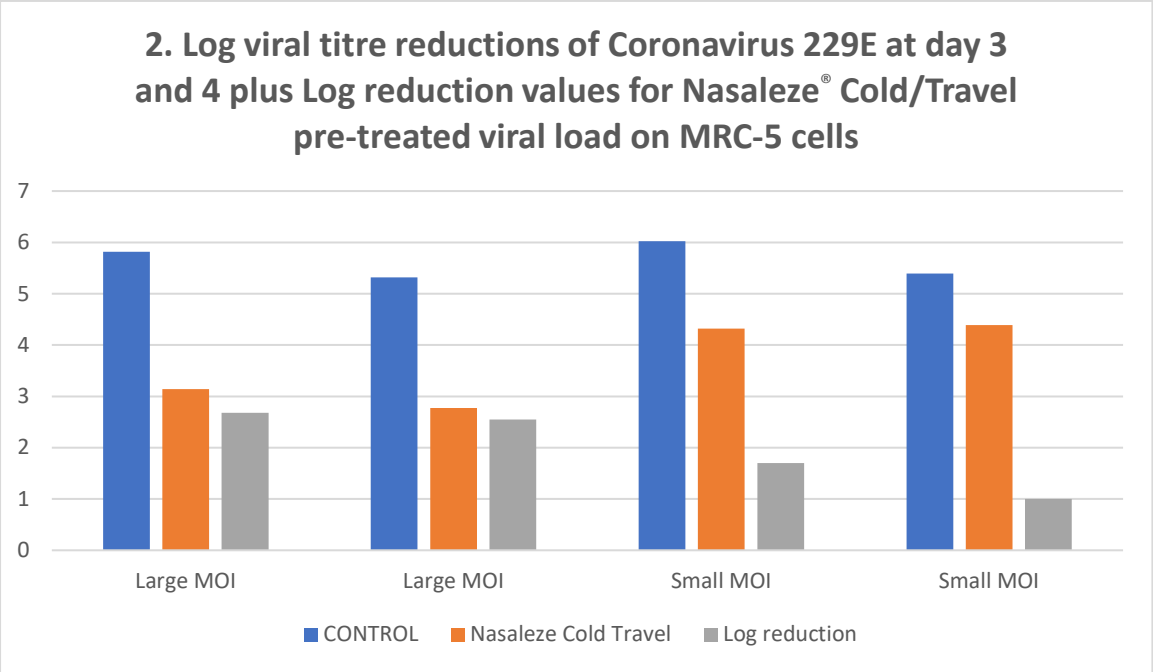


Following a 3 and 4 day incubation period with a high MOI of CoV 229E the negative control resulted in an average viral titre of 5.82 ± 0.35 $Log_{10}TCID_{50}$/mL and 5.32 ± 0.35 $Log_{10}TCID_{50}$/mL, respectively. Pre-treatment of MRC-5 cells with Nasaleze® Cold/Travel resulted in a 2.68 $Log_{10}TCID_{50}$/mL and 2.55 $Log_{10}TCID_{50}$/mL reduction in viral titre on day 3 and day 4 post-infection, respectively, when compared to the negative control showing an average of 3.14 ± 0.18 and 2.77 ± 0.53 Table 3 and 4 - Chart 2.

Large viral titre

Product	Average Viable CoV 229E ± SD ($Log_{10}TCID_{50}$/mL)		Log Reduction ($Log_{10}TCID_{50}$/mL)	
	Day 3	Day 4	Day 3	Day 4
Negative Control	5.82 ± 0.35	5.32 ± 0.35	N/A	N/A
Nasaleze® Cold/Travel	3.14 ± 0.18	2.77 ± 0.53	2.68	2.55

Table 3. Log TCID50 and Log reduction values for human coronavirus 229E (CoV 229E) following treatment with Nasaleze® Cold/Travel before infection at a high multiplicity of infection and incubated for 3 and 4 days. N/A = not applicable, SD = standard deviation.



Small viral titre

Following a 3 and 4 day incubation period with a low MOI of CoV 229E the negative control resulted in an average viral titre of 6.02 ± 0.53 $Log_{10}TCID_{50}$/mL and 5.39 ± 0.18 $Log_{10}TCID_{50}$/mL, respectively. Pre-treatment of MRC-5 cells with Nasaleze® Cold/Travel resulted in a 1.70 $Log_{10}TCID_{50}$/mL and 1.00 $Log_{10}TCID_{50}$/mL reduction in viral titre on day 3 and day 4 post-infection, respectively, when compared to the negative control (Table 4, Chart 2).

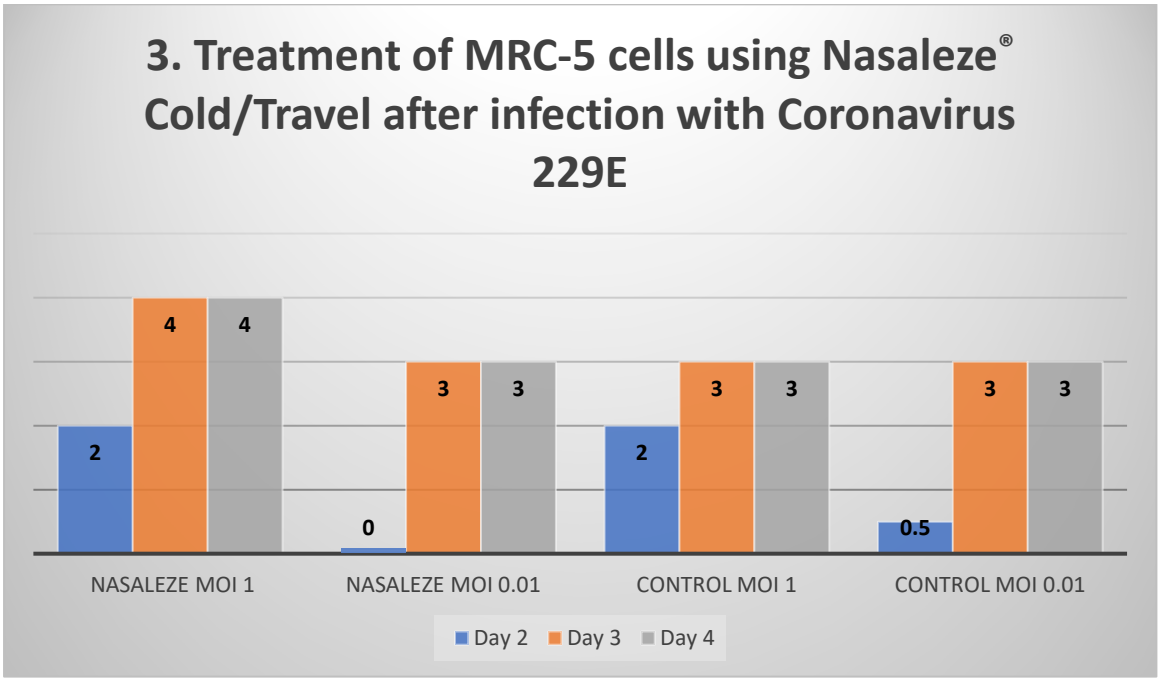
Product	Average Viable CoV 229E ± SD ($Log_{10}TCID_{50}$/mL)		Log Reduction ($Log_{10}TCID_{50}$/mL)	
	Day 3	Day 4	Day 3	Day 4
Negative Control	6.02 ± 0.53	5.39 ± 0.18	N/A	N/A
Nasaleze® Cold/Travel	4.32 ± 0.35	4.39 ± 0.18	1.70	1.00

Table 4. Log TCID50 and Log reduction values for human coronavirus 229E (CoV 229E) following treatment with Nasaleze® Cold/Travel before infection at a low multiplicity of infection and incubated for 3 and 4 days. N/A = not applicable, SD = standard deviation.

Treatment capabilities of Nasaleze® Cold/Travel

Cytopathic effect of CoV 229E on MRC-5 cells treated with Nasaleze® Cold/Travel after viral infection

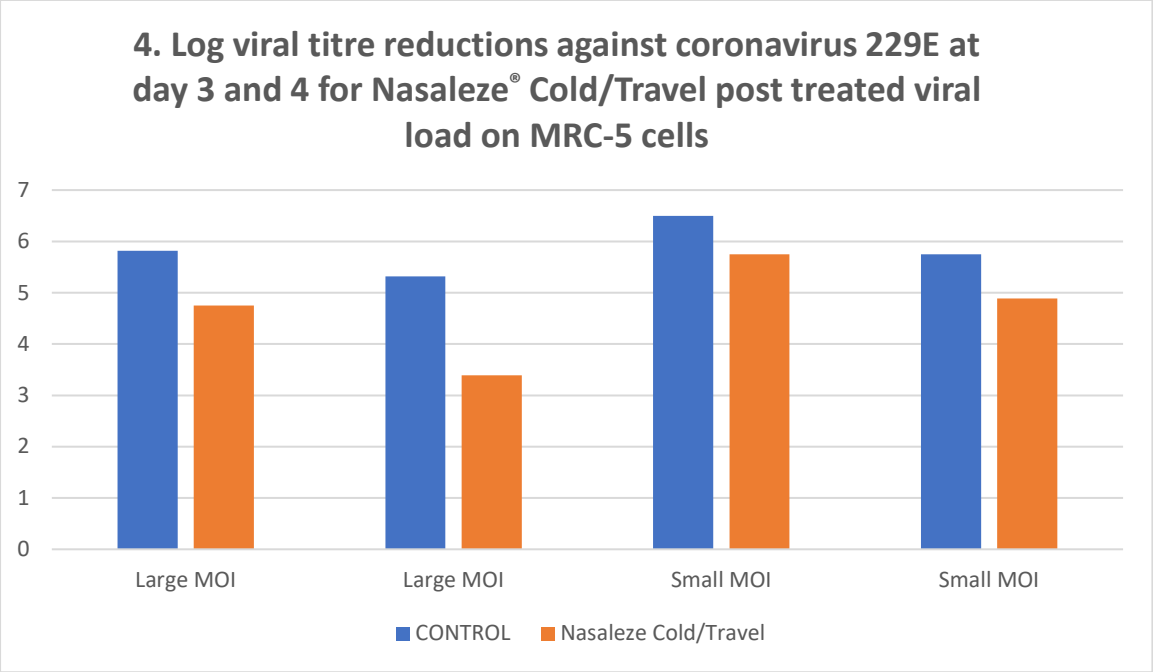
Following a 2, 3 and 4 day incubation period, the CPE of the test plate was scored. Representative images of the CPE observed are presented in Appendix II. Duplicate cells treated with Nasaleze® Cold/Travel after infection with a high MOI of CoV 229E showed mild CPE on day 2 and severe CPE on days 3 and 4 post-infection. Duplicate cells treated with Nasaleze® Cold/Travel after infection with a low MOI of CoV 229E showed no CPE on day 2 and moderate CPE on days 3 and 4 post-infection.



Viral titration of samples treated with Nasaleze® Cold/Travel Blocker after viral infection Chart 4

Following a 3 and 4 day incubation period with a high MOI of CoV 229E the negative control resulted in an average viral titre of 5.82 ± 0.35 $Log_{10}TCID_{50}$/mL and 5.32 ± 0.35 $Log_{10}TCID_{50}$/mL, respectively. Treatment with Nasaleze® Cold/Travel after infection resulted in a strong log reduction on days 3 and 4 at 4.75 ± 0.00 and 3.39 ± 0.18 respectively.

Furthermore a 3 and 4 day incubation period with a low MOI of CoV 229E the negative control resulted in an average viral titre of 6.50 ± 0.00 $Log_{10}TCID_{50}$/mL and 5.89 ± 0.18 $Log_{10}TCID_{50}$/mL, respectively. Treatment with Nasaleze® Cold/Travel after infection resulted in a strong log reduction on days 3 and 4 at 5.75 ± 0.00 and 4.89 ± 0.18 respectively.



Discussion

The dissemination of potentially pathogenic viruses increases infection risk in both healthy and immunocompromised individuals. Coronaviruses are enveloped, single stranded RNA viruses responsible for a variety of upper-respiratory tract illnesses in humans. The severity of these illnesses ranges from mild as in common cold to severe acute respiratory syndrome as seen in the recent COVID-19 pandemic. Coronaviruses are thought to be predominantly transmitted through respiratory droplets with some evidence to suggest the virus can remain active on fomites for several days. Interventions, both preventative and curative, are essential to slowing and/or stopping the spread of coronaviruses.

The assessment of interventions against coronavirus surrogate strains allows for the safe evaluation of product efficacy. Coronavirus 299E is structurally and genetically similar to the SARS-CoV-2 virus. Since the COVID-19 pandemic, the Australian regulatory body, Therapeutic Goods Administration (TGA), is the first regulatory body to announce that **Coronavirus 229E as a suitable coronavirus surrogate strain for biocide coronavirus claims.**

Two different experiments were performed to investigate the anti-viral efficacy of Nasaleze® Cold/Travel. In the first experiment, MRC-5 cells were pre-treated with Nasaleze® Cold/Travel before infection with high and low doses of CoV 229E. in the second experiment MRC-5 cells were infected with a high and low dose of CoV 229E before treatment with Nasaleze® Cold/Travel. Treatment with Nasaleze® Cold/Travel did not damage the experimental MRC-5 cell line, but yielded substantial reductions in viral titre indicating a high level of anti-viral potential. Although the reduction in CPE was not large or maintained it should be noted that a sub optimal dose was used representing only one dose into a single nostril, whereas real life clinical data accumulated thus far has shown that a three times daily dose into each nostril can significantly reduce airborne infection.[1,2,3]

Future work could investigate the optimal dosing of Nasaleze® Cold/Travel, simulating the real-life intended use of the product. Additionally, similar experiments could be performed using other respiratory viruses such as influenza, adenovirus and rhinovirus. Finally, as this formulation shows such promise in both preventing and treating viral infection, a 3D primary nasal cell culture model could be considered for use to obtain a more translatable result as well as clinical evaluations in human subjects to add to the existing database

for this unique powder cellulose, signalling agent and garlic extract, marketed as Nasaleze® Cold & Flu Blocker and Nasaleze® Travel.

Key take away points from the report

We asked co-author Peter Josling for his comments on the results…

"This is a very interesting *in vitro* study that clearly shows Nasaleze® Cold & Flu Blocker and Nasaleze Travel are unique active formulations in the fight to both prevent and treat coronavirus infections.

It is clear that pre-treatment reduces viral replication and may therefore stop Coronavirus 229E in its tracks when used at optimum dosing levels.

Even when viral replication is already infecting healthy human cells Nasaleze® Cold/Travel can attack and disable viral replication.

These results are from a SINGLE dose of Nasaleze® Cold/Travel and we would expect multiple doses to be even more effective.

Nasaleze® Cold/Travel do not have any negative cytopathic effect on human cells.

This is a step forward in the prevention and management of coronavirus infection."

Dr Peter Josling
Herbal Research Centre

References

1. Preventing airborne infections with an intra nasal cellulose powder formulation, Hiltunen R, Josling P D, James M, Advances in Therapy 2007, 24/5 – 1146-1153
2. Use of Nasaleze® Cold as prevention method for acute respiratory illness in paediatrics, Geppe N A, Farber I M, Kozhevinkova T N, Andriyanova E V, unpublished data on file
3. Virucidal activity of Nasaleze® and Nasaleze® Cold/Travel in cell cultures infected with pathogenic avian flu virus H5N1, Lvov D K, Deryabin P G, European Journal for Nutraceutical Research 2010

Project Start Date 20th April 2020

Project Completion Date 3rd July 2020

Appendix 1

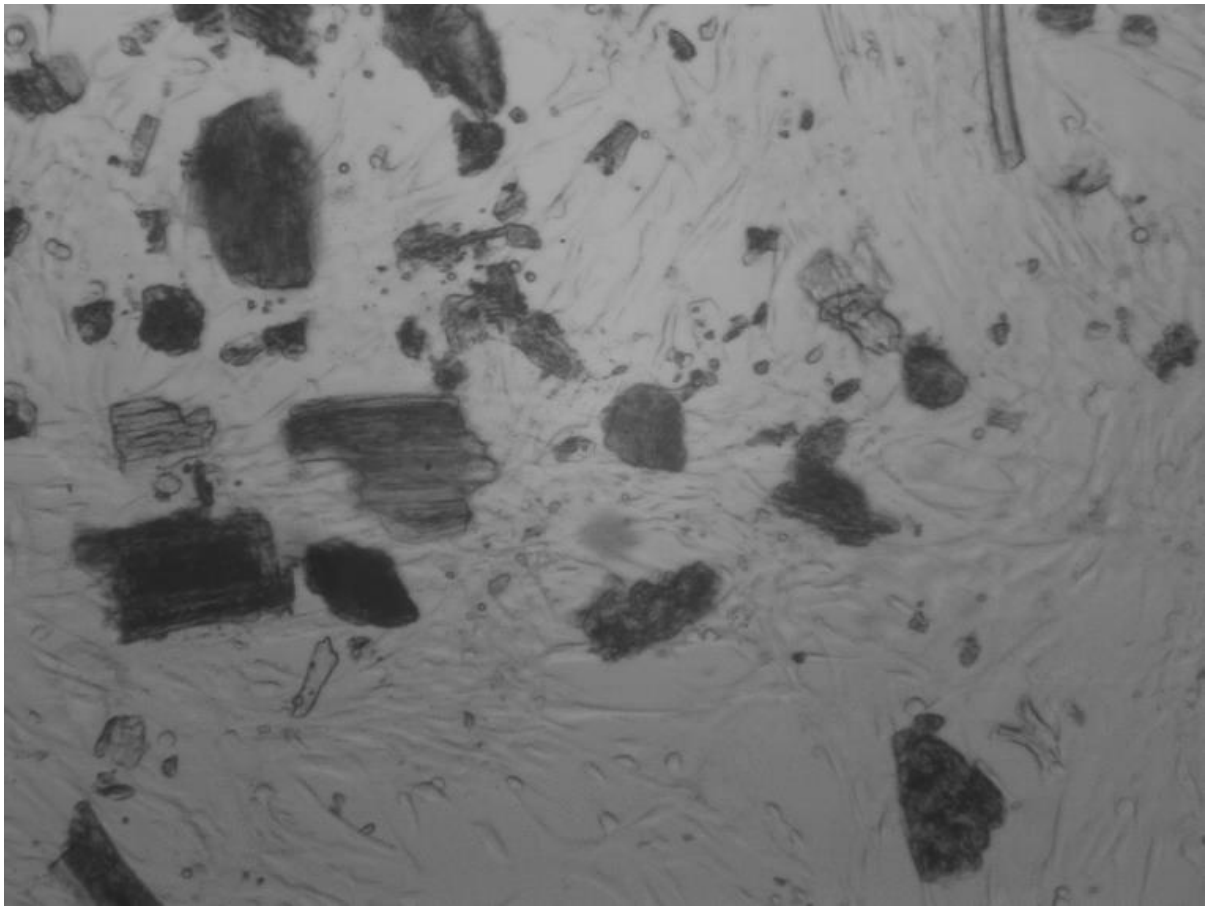


Figure A. Microscope images showing the residue visible on MRC-5 cells after treatment with Nasaleze® Cold/Travel.

Appendix II

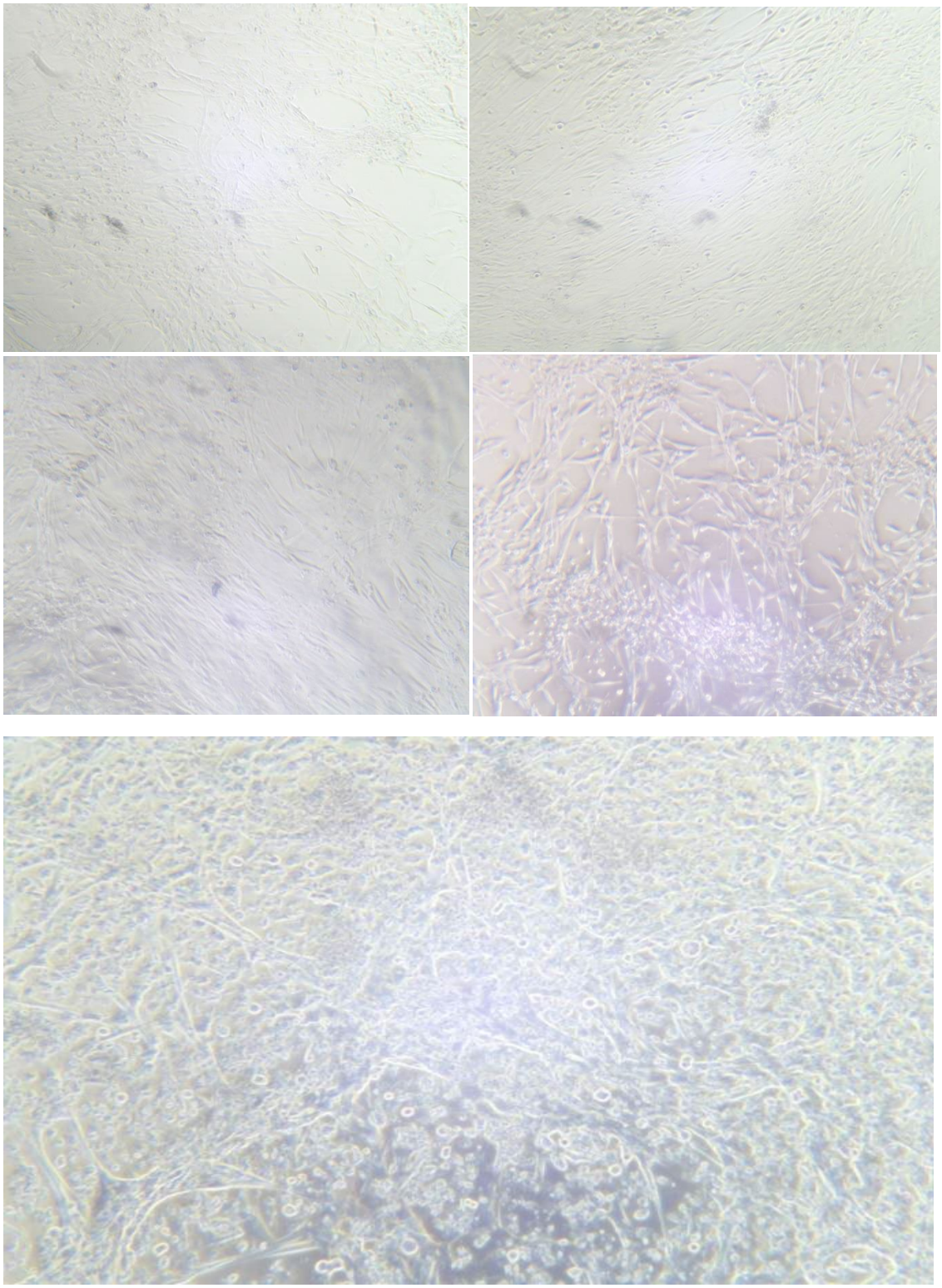


Figure B: Representative images of cytopathic effect of MRC-5 cells observed throughout the study. Top left = No CPE, Top right = Slight CPE, Middle left = Mild CPE, Middle right = Moderate CPE, Bottom = Severe CPE.

Press release

New study highlights the potential role for Nasaleze® Cold & Flu Blocker and Nasaleze® Travel for protecting against Coronavirus 229E

10th September 2020

New results from an in-vitro study have highlighted the potential viral efficacy of the patented cellulose powder formulation, found in found in Nasaleze Cold/Travel, in protecting against the Human Coronavirus (CoV) 229E.

In the first section of the study, MRC-5 cells (Medical Research Council cell strain 5) were pre-treated with Nasaleze before infection with high and low doses of CoV 229E. In the second section of the study, MRC-5 cells were infected with high and low doses before treatment with Nasaleze. The virucidal activity of the formulation was measured at 48, 72 and 112 hour periods after incubation. Results from both experimental arms of the study showed strong reductions in viral titre of Coronavirus 229E compared to a control virus titre.

Taking place between 20th April and the 3rd July 2020, the study was conducted by Perfectus Biomed at their microbiological testing facility in the UK and was supervised from afar by Professor Ted Popov from the University Hospital St. Ivan Rilski in Bulgaria.

Commenting on the research, Professor Ted Popov said: “The nose is effectively the "Achilles heel" of the human body during a SARS-CoV-2 invasion. It has an abundance of Angiotensin Converting Enzyme 2 (ACE2) receptors which are targeted by the virus as a gateway for the infection. Blocking the viral access to those receptors by a mechanical barrier is a powerful tool to help prevent the infection.”

Professor Popov continues: “This research shows that minimal amounts of a natural compound has virucidal activity on a Corona viral strain. It shows the role a powder methylcellulose can play in creating the ultimate barrier inside the nasal cavity, shielding the plethora of ACE2 receptors there. The study suggests methylcellulose can play a complementary role along face masks to strengthen the nose’s barrier function and provide added protection to individuals.”

Nasaleze Nasal Spray products provide protection from airborne germs and viruses by coating the lining of the nose. The powder turns to a gel in the nose, creating a physical barrier. This research further reinforces the antiviral activity of the product formulation and the preventative role it can play to

disrupt viral replication, alongside important steps such as washing your hands regularly, covering your face, and maintaining social distancing.

This study follows numerous previous studies into the effectiveness of Nasaleze, including a study published in the European Journal for Nutraceutical Research that showed the product's ability to combat the avian flu virus H5N1 and prevent it from continuing to replicate in human cell lines.

Coronaviruses are responsible for a variety of upper-respiratory tract illnesses in humans, ranging from mild conditions such as the common cold to severe acute respiratory syndrome as seen in the recent COVID19 pandemic. Coronaviruses are thought to be predominantly transmitted through respiratory droplets.

ENDS

Notes to Editors:

Professor Todor (Ted) A. Popov: Having graduated in medicine from the Medical University in Sofia, Bulgaria, he is currently a Professor at the University Hospital 'Sv. Ivan Rilski' in Sofia. His areas of interest include asthma diagnosis and treatment, allergic rhinitis, non-invasive methods for assessment of airway inflammation, and bronchial reactivity, as well as the planning and design of research studies. He has a rich publishing history and is member of the editorial board and reviewer of international and Bulgarian medical journals.

Perfectus Biomed: A leading GLP compliant and UKAS accredited Contract Research Organisation. They provide both standard and customised microbiological testing services, with extensive experience of developing 'fit for purpose' experiments that mimic 'real-life' scenarios.

UK market planned Autumn 2020 social media promotional campaign for Nasaleze Cold & Flu Blocker and Nasaleze Travel

Nasaleze®

1. WASH, SPRAY, MASK

Nasaleze®

Positions the product as an additional preventative measure.

2. AN EXTRA LAYER OF PROTECTION

Nasaleze®

Nods to the physical action of the product. Highlights the end goal – and the associated peace of mind.

3. BACK TO SCHOOL

Nasaleze®

‘Send them back to school with an extra layer of protection.’

Targeted at parents and bloggers.

4. KEEP SAFE FROM GERMS AND VIRUSES

Nasaleze®

Using up to date clinical data we are now able to utilise statements around protection from germs and viruses.

Alongside the powerful action of ‘keep safe’ we are giving the consumer clear direction on the use of this product.

Additional Nasaleze® Cold/Travel Clinical Trial Data

NO.	STUDY	POPULATION	DESCRIPTION	MEASUREMENTS AND RESULTS
1	Preventing Air-Borne Infections with an Intranasal Cellulose Powder Formulation *Hiltunen R, Josling PD, James MH* Advances in Therapy. 2007; 24(5):1146-53.	N=52 subjects	• Randomized = Nasaleze Cold vs. Nasaleze Allergy. • Determine whether Nasaleze Cold (with garlic) can reduce airborne infections. • Study period = 8 weeks in Finland & United Kingdom. • DOSE = 1 puff of Nasaleze Cold per day. Increase to 3 puffs if subject became sick with. infection. • Diary was kept recording 5-point symptoms scale.	Nasaleze Cold vs. Nasaleze Allergy: • Significantly fewer infections: 20 vs. 57 p<.001 • Far fewer days with infection: 126 vs. 240 days p<.05 • Number of serious infections > 7 days 6 vs. 12 p<0.05
2	Use of Nasaleze Cold as Prevention Method for Acute Respiratory Illnesses in Pediatrics *Geppe NA, Farber IM, Kozhevnikova TN, Andriyanova EV* Pharmateka, No: 14, 2010, p.60-65 New discovery of this data being published.	N=60 Children ▪ N=40 Treatment ▪ N=20 Control	• Randomized = Nasaleze Cold vs. No Treatment • Determine whether Nasaleze Cold can reduce incidence of Upper Respiratory Tract Infection (URTI) • Study period = 6 weeks in Moscow. • Use of 4-point symptoms scale • ENDPOINTS ▪ Incidence of Illness (URTI) ▪ Symptom Scores	Nasaleze Cold vs. No Treatment NZ Cold / Control / p-value • Did not fall ill at all 80% 0% p < 0.05 • Fell ill once 15% 55% NS • Fell ill twice 5% 45% p < 0.05 DEC 2008 - FEB 2009 (No Treatment) / DEC 2009 - FEB 2010 (Nasaleze Cold) • Incidence of URTIs 2.7 0.3 • Duration of URTIs 7.7 days 3.2 days Symptom Scores • In TREATMENT Group, there was definite reduction in URTI symptoms from Week 2 to Week 6
3	Viricidal Activity of Nasaleze and Nasaleze Cold in Cell Cultures Infected with Pathogenic Avian Flu Virus (H5N1) *Lvov DK, Deryabin PG* European Journal for Nutraceutical Research www.phytomedcentral.org | March 24, 2010	In vitro	TEST ARTICLES: Nasaleze Allergy; Nasaleze Cold CELL CULTURES: Porcine Embryo Kidney Cell Cultures (SPEV) INOCULUM: Flu A/H5N1 Virus ▪ High Dose = 10.0 TCID50 ▪ Low Dose = 1.0 TCID50 PROTOCOL: VARIATION 1 (Preventive; Before Infection with Flu Virus) / VARIATION 2 (Medical + Preventive; Immediately After Infection with Flu Virus) CONTROLS: CONTROL 1 (Flu Virus ✓, Nasaleze X) / CONTROL 2 (Flu Virus X, Nasaleze ✓) / CONTROL 3 (X, X) EVALUATIONS • Timepoints: 2 days → 3 days → 4⅔ days • Assessments ▪ Viral Activity: Concentration of Flu Virus -- determined through titration for infectious activity ▪ Efficacy: % Survival of Infected Cells -- determined using optical microscopy to evaluate cells for morphology, vitality, cytoproliferative activity	VARIATION 1 -- PREVENTIVE Antiviral Activity BEFORE Infection with H5N1 Flu Virus ENDPOINT = CELL SURVIVAL FLU VIRUS HIGH DOSE: 2 days → 3 days → 4⅔ days Nasaleze Cold 100% 75% 0% Nasaleze Allergy 100% 20% 0% NO Test Product 80% 5% 0% FLU VIRUS LOW DOSE: 2 days → 3 days → 4⅔ days Nasaleze Cold 100% 100% 0% Nasaleze Allergy 100% 85% 0% NO Test Product 95% 30% 0% VARIATION 2 -- MEDICAL + PREVENTIVE Antiviral Activity IMMEDIATELY AFTER Infection with Flu ENDPOINT = CELL SURVIVAL FLU VIRUS HIGH DOSE: 2 days → 3 days → 4⅔ days Nasaleze Cold 100% 80% 0% Nasaleze Allergy 100% 25% 0% NO Test Product 75-80% 5% 0% FLU VIRUS LOW DOSE: 2 days → 3 days → 4⅔ days Nasaleze Cold 100% 90% 0% Nasaleze Allergy 100% 85% 0% NO Test Product 95% 25-30% 0% VARIATIONS 1 & 2 -- PREVENTIVE vs. MEDICAL + PREVENTIVE ENDPOINT = FLU VIRUS TITERS (at 3 Days) PREVENTIVE / MEDICAL + PREVENTIVE Nasaleze Cold 1.5 3.0 Nasaleze Allergy 3.0 4.0 NO Test Product 7.5 7.5
4	Nasaleze & Nasaleze Cold Safety Study Study of Effects of Inert Cellulose Powder on Nasal Mucosa *Angotoyeva IB and Sukhovetchenko YV* Russian Allergological Journal. N6; 2011.	N=60 ▪ N=30 GROUP I ▪ N=30 GROUP II	• GROUP I = Healthy Volunteers ⇒ Nasaleze Cold • GROUP II = Allergic Rhinitis ⇒ Nasaleze • ENDPOINTS ▪ Evaluation of Nasal Mucosa Condition ⇒ Assessed by Rhinoscopy + Endoscopy ▪ Mucociliary Clearance (MCC) ⇒ Assessed using Saccharine Test + Methylene Blue ▪ Ciliary Beat Frequency (CBF) ▪ Cytological Analysis of Mucosal Smears ▪ Signs of Inflammation ▪ Quality of Life (questionnaire)	• Rhinoscopy + Endoscopy + Cytology showed attenuation in nasal mucosa inflammation • Mucociliary transport was not inhibited • Nasaleze had no ciliotoxic effect on nasal mucosa • Nasal mucosa cell composition was unaffected Nasaleze Product Safety • No allergic reactions or significant side effects Quality of Life • QoL significantly improved in subjects with AR
5	Evaluation Biological Activity of Allicin + Nasaleze *Cutler RR, PhD* Unpublished / Data on File	In vitro	Bacteria: MRSA Clinical Isolate Test Samples: Allicin Powder; Nasaleze Cellulose Powder Control: Gum Acacia Powder Methods: Test sample is spread on 6mm well of plate / Incubated / Observed for Zone of Inhibition Zone of Inhibition / Biological Activity / Clinical Correlate No Zone: X: Bacterial Resistance Zone Size > 12mm: ✓: Bacterial Sensitivity	# / TEST SAMPLE / Allicin/Cellulose Ratio / 100 µg / 150 µg 1 Control Control Powder Alone 0 0 2 Nasaleze Cellulose Powder Alone 0 0 3 Allicin BN 2069 Allicin Powder Alone 14 19 4 Allicin + CPC 2102 4:1 23 27 5 Allicin + CPC 2102 6:1 28 28 6 Allicin + CPC 2069 4:1 12 17 7 Allicin + CPC 2102 8:1 22 26

In a clinical in vitro study “Virucidal activity of Nasaleze Cold/Travel in cell cultures infected with pathogenic avian flu virus (H5N1)”...

“It was proven that Nasaleze Cold/Travel was capable of significantly reducing the production of the flu A/H5N1 virus by infected cells over a period of 72 hours from just a single daily dose.”

“The data suggests that Nasaleze Cold/ Travel could be used as a preventative measure and a treatment option for this persistent viral infection.”

1Lvov DK, Deryabin PG. Virucidal activity of Nasaleze Travel in cell cultures infected with pathogenic avian flu virus (H5N1 ” conducted at the Ivanovsky State Scientific Research Institute of Virology at the Russian Academy of Medical Sciences, Moscow.

Nasaleze®

Nasaleze International Ltd,
Nunnery Mills, Old Castletown Road, Douglas,
Isle of Man, IM2 1QA, British Isles

E: info@nasaleze.com
T: +44 (0) 1624 611 050

www.nasaleze.com