COVISPRAY

Introduction: Covid-19 is a multifactorial disease caused by Corona virus and nearly 90% of Covid-19 infections occur through virus entry in the nasal cavity. The virus starts attacking the nasal mucosa cells as the concentration of virus attaching ACE2 receptors is very high on the nasal mucosa.

The nasal mucosa is a multiple layer of epithelial cells with connective tissue in between the cells to form an intact nasal barrier.

The Covid-19 virus surface contains many protruding SPIKES which are glycoproteins. The S1 spike and a RBD or Receptor Binding Domaine protein, bind to the ACE-2 receptors situated on the nasal mucosa cells.

This allows virus entry into the cells, virus starts growing, and a huge quantity of newly generated free virus particles are then liberated on the nasal mucosa surface. These viruses attack new healthy cells, and open gates for virus entry into the circulation.

The immune cells, particularly the mast cells, B & T lymphocytes get activated and trigger inflammatory reaction by liberating multiple cytokines, particularly the interlukine-6 and TNF-alpha to fight the infection.

The immune system tries to control the infection but if the infection continues, it gets tired, fatigued, stressed and finally there is burn-out.

When symptoms start appearing, the disease pathogenesis is already installed, and virus is already disseminated in the body. The key disease symptoms include cough, fever, respiratory difficulties, loss of taste & smell, body pain, sore throat, eye irritation, and headache, followed by severe respiratory distress in the late phase of the disease. Therefore, to reduce the consequences of infection, we must act at a time on all these parameters but there is no such treatment yet available.

Currently available treatments: For the time being, there is no treatment against Covid-19. A new generation of RNA vaccines are introduced recently but their long-term efficacy and side-effects are not known. Nasal wash with water, saline or salt solutions may help reduce free virus particles concentration on the nasal mucosa but this is not very effective as virus continues growing in nasal mucosa cells and continues generating new virus particles. In addition, such a treatment must be used very frequently. A few nasal solutions containing thickening agents (ex. carrageenan) are also available, but they have no specific anti-viral or anti-inflammatory properties, and such solution films are not stable leading to their disintegration within a few minutes. The cotton / polyester nasal masks minimize dissemination of virus particles in the air, but they do not offer total protection against virus entry. These masks neither help to reduce free virus particles which are continuously generated on the nasal surface in already infected individuals nor do they help in reconstituting damaged nasal mucosa which lets free virus entry into the circulation.

The efficacy of the vaccine and duration of protection offered is also not yet clear. Recent observations in the city of Manaus in Brazil where 76% residents were seropositive in summer 2020, following a new surge of widespread Covid-19 infection since December 2020 let unanswered questions about herd immunity and the vaccine efficacy against new emerging Covid-19 variants (See: *Ester C Sabino et al.* ***Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence***. *Lancet report: January 27, 2021DOI:https://doi.org/10.1016/S0140-6736(21)00183-5*).

Therefore, its highly important to find a multitarget treatment which can minimize simultaneously virus infection, inflammation, and nasal mucosa damage to reduce immune stress and the development of severe respiratory symptoms.

Conception of Covispray

As “solid” bandages cannot be applied directly on the nasal mucosa, after 20-years of R&D, Vitrobio developed a stable, osmotically active, liquid bandage for direct application on the nasal surface. COVISPRAY is a glycerol based, osmotic, filmogen solution, containing 2 jellifying agents and a few specific dual acting polymers. Covispray was conceived in 2020 but since 1999, Vitrobio has been conducting R&D to find an osmotic liquid filmogen bandage which can be applied on the surface of hidden or difficultly accessible tissues. Vitrobio has already acquired experience in conceiving medical devices to treat rhino & influenza virus infections. These filmogen medical devices are marketed since 2014. Material vigilance data gathered on millions of patients during this period proves good efficacy and high safety of glycerol containing polymeric medical devices. The experience acquired with these products explains why we could apply the same technology to Covid-19 infection.

All Vitrobio filmogen osmotic bandages contain glycerol, a highly osmotic solution, capable of attracting hypotonic liquid & generating a strong osmotic flow, when applied on a live biological membrane. The liquid flow mechanically detaches & drains all the free contaminants (virus particles, inflammatory cytokines) which are present on such biological surfaces (Patent 2000-2005).
Unfortunately, the liquid flow also dilutes glycerol and rapidly reduces its osmotic potential. Therefore, a few specific glycerol molecules binding plant polymers were added in minimum concentrations, just sufficient to block all the glycerol molecules. Glycerol – polymer binding helps to render the glycerol filmogen and mechanically resistant to liquid flow. Such a glycerol film can remain for at least 4-6h on the nasal mucosa.
Polymers, for example plant tannins, are very big and inert molecules having multiple binding sites for macromolecules and proteins. This is why traditionally tannins or polymers are used to block skin proteins to convert it into leather. We selected some specific dual acting polymers which always bind to glycerol and render the glycerol film resistant to osmotic flow, while other free sites of these polymers bind with specific proteins like Covid-virus S1 glycoprotein, RBD protein, and Covid-19 specific IL-6, TNF-α, IL-10, IL-13, & GM-CSF pro-inflammatory cytokine.
Mode of action: When Covispray is spayed on the nasal mucosa, it forms instantly a stable osmotic film. Osmosis creates a strong hypotonic liquid flow from the nasal tissue, swells the jellifying agents and renders the film absorbent. In addition, the osmotic liquid flow cleans the nasal surface by detaching and draining the contaminants present on the nasal mucosa.
The film also acts like a physical barrier on the nasal surface to prevent encounter of incoming virus particles with the nasal mucosa. When the nasal mucosa is clean, inflammation starts reducing which offers a good environment for cells to grow and reconstitute natural, intact, nasal defensive barrier.
The aim of Covispray treatment is not to cure the disease immediately but simply to reduce the concentration of virus & inflammatory cytokines on the nasal mucosa to allow reconstitution of the natural nasal mucosa barrier. A healthy mucosa barrier can defend against infection, reduce the systemic entry of contaminants, inflammatory cascade, and in consequence immune stress & the chances of immune burn-out.
Although the Covispray film continuously cleans the contaminants from the nasal mucosa, the circulating viruses also continue attacking their site of predilection (nasal mucosa) & pouring newly generated viruses on the nasal surface. Therefore, up to the time the viruses have not

totally disappeared from the body, the naso-pharyngeal PCR test should remain positive. Similarly, due to the virus presence in the body, minor clinical signs, particularly the respiratory symptoms, may also persist with minimal intensity.
The use of Covispray should not replace any recommendation directed to avoid disease progression but should be considered as a complement to existing measures.
Performance studies: Among the glycerol binding polymers, we selected those which can bind with glycerol as well as with the Covid-19 S1 spike and covid-19 RBD proteins, which allow virus entry into the cells.
The summaries of the key parameters are given below:

(1) Selection of natural polymer or polymeric associations to render osmotic glycerol filmogen and resistant to mechanical pressures

To evaluate the polymer-glycerol molecule binding, minimum concentrations of each plant extract, either alone or in association, were added in glycerol. The solution (20 µl) was spread as a film on live human multicellular growing epidermal surfaces in vitro. Normally, the cells are kept on one sponge to nourish the cells with osmotic liquid (culture medium) flow. If one extra sponge is incorporated, the distance between the liquid culture medium and the cells increases with poor medium supply to the cells. In 2 sponge control cells with no product application, cells should die within 2-3 days due to dehydration. The epidermis was removed and stirred in a liquid medium every day to exert mechanical pressure on the film. If the plant tannin binds with glycerol molecules, the film is not disintegrated and continues attracting osmotic liquid to keep the cells hydrated. Cellular viability is quantified and is proportional to the duration of film retention on the epidermal membrane.
Among the glycerol binding plant extract, good film resistance was obtained by adding an association of 4 natural plant polymers (CsL + ClR + UdP + TpF) at a concentration <1.0% compared to the controls. Key results are shown in Table 1.

Test product	**% cell survival ± S.D. (mean of n=6)**	**% increase or decrease in cell survival or osmosis versus positive control**
Negative control (1-sponge)	96.5 ± 6.36	Standard cultures
Positive control (2- sponges)	8.52 ± 1.36	Nearly all dead cells
Test products with each polymer at a concentration of 0.10%		
10% Gly alone (glycerol control)	26.34 ± 3.27	+ 17.82
10% Gly + CsL + ClR	80.32 ± 6.08	+ 71.80***
10% Gly + CsL + UdP	69.76 ± 5.10	+ 61.24***
10% Gly + CsL + TpF	89.55 ± 6.22	+ 81.03***
10% Gly + CsL + ClR + UdP	81.32 ± 4.65	+ 72.80***
10% Gly + CsL + ClR + Hh	76.45 ± 6.79	+ 67.93***
10% Gly + CsL + ClR + TpF	69.38 ± 5.71	+ 60.86***
10% Gly + Csl + ClR + UdP + TpF	93.95 ± 6.54	+ 85.43***

*Table 1 : % increase (+) or decrease (-) in cell survival of epidermal cells in 2-sponges cellular dehydration model when exposed to 10% glycerol containing 0.10% glycerol binding polymer(s) versus 10% glycerol alone. The cell membranes were exposed to mechanical pressure at 6h, 24h, and 48h by stirring in culture medium. Higher cell survival indicates higher film resistance to mechanical pressure and better filmogenicity. Gly: glycerol, HpP: Hydroxy propyl cellulose, Sg: Solagum as an association of acacia and xanthan gums. *p<0.05 **p<0.01 ***p<0.001 v/s 10% glycerol alone*

(2) Determination of polymer binding with Covid-19 S1 glycoproteins and viral RBD proteins

For this study, each plant extract and excipient used in the preparation of Covispray were tested in 2 concentrations, close to the concentrations used in the finished Covispray (Coded as PIRDAL) composition, employing the ACE2:SARS-CoV-2 Spike S1 or RBD Inhibitor Screening Assay Kits (BPS Bioscience, ref. 79945, lot 201001-K). Due to technical constraints, the final Covispray concentrations tested were 5% and 10%.

These studies were performed by Tebu-bio (USA-French division). Both kits contained a 96-well microplate, purified ACE2 and SARS-CoV-2 Spike S1 or RBD proteins, Streptavidin-HRP or HRP labelled anti-human Fc region antibodies, and assay buffers 2 or 1 for 100 binding reactions. The kits were designed to detect Covid-19 Spike S1-Biotin protein by Streptavidin-HRP or Fc-tagged RBD spike proteins. ACE2 protein was attached to a nickel-coated 96-well plate. Next, SARS-CoV2 Spike S1-Biotin or spike-RBD-Fc were incubated with ACE2 on the plate. Finally, the plate was treated with Streptavidin-HRP or Anti-Fc-HRP, followed by addition of an HRP substrate to produce chemiluminescence, which was measured using a chemiluminescence reader. All experiments were conducted at room temperature.

According to the solubility of test products, a low and a high concentration was tested as follows: solagum (Sg) excipient negative control (0.05 and 0.10%), hydroxypropyl cellulose (HpP) excipient negative control (0.10 & 0.30%), HhP plant extract test control (0.10 and 0.30%), Covispray individual polymer CsL, ClR (0.05 and 0.15%), UdP and TpF (0.10 & 0.30%), association of 4 Covispray polymers (0.05 and 0.15%), and finished Covispray formulation (5% and 10%). Polymeric association called S1cyanidins (CsL, ClR, UdP, TpF) are derived from tannins rich whole plant extracts. Due to technical difficulties, it was not possible to test above 10% Covispray finished formulation.

Results of S1 & RBD protein properties of Covispray plant polymers:

The positive PBS controls have no effect, neither on the binding of Covid-19 S1 protein (Fig.2AB) or RBD proteins (Fig.2CD). Similarly, no statistically significant inhibition of S1 or RBD proteins was observed with Sg and HpP excipients, HhP plant extract control, or ClR, UdP, and TpF individual polymers although slight RBD protein binding was observed with 0.30% TpF and HhP polymers. On the contrary, CsL at a concentration of 0.05% blocked nearly 60% S1 spike protein while a slightly higher concentration (0.15%) neutralizes above 90% S1 spike protein (both $p<0.001$, Fig.2AB). The same polymer, at concentrations of 0.05% and 0.15% also blocked nearly 70% and 95% RBD proteins ($p<0.001$, Fig.2CD). It should be noted that the maximum concentration of CsL + ClR + UdP + TpF which could be tested was 0.15%, nearly 3-times lower than what is incorporated in the finished composition. The Covispray final composition at a concentration as low as 5.0%, blocked on average 50.6% S1 protein and 49.6% RBD protein while at 10% concentration the inhibition was 72.2% for S1 and 72.0% for RBD protein, compared to positive PBS controls. It is comprehensible that RBD being a fragment protein of SARS-CoV-2 S-protein, shows similar polymeric binding with both proteins. These results clearly show that Covispray at a concentration of only 10% can block up to 70% of Covid-19 S1 and RBD proteins which are involved in virus entry into the host cells through ACE-2 receptor binding.

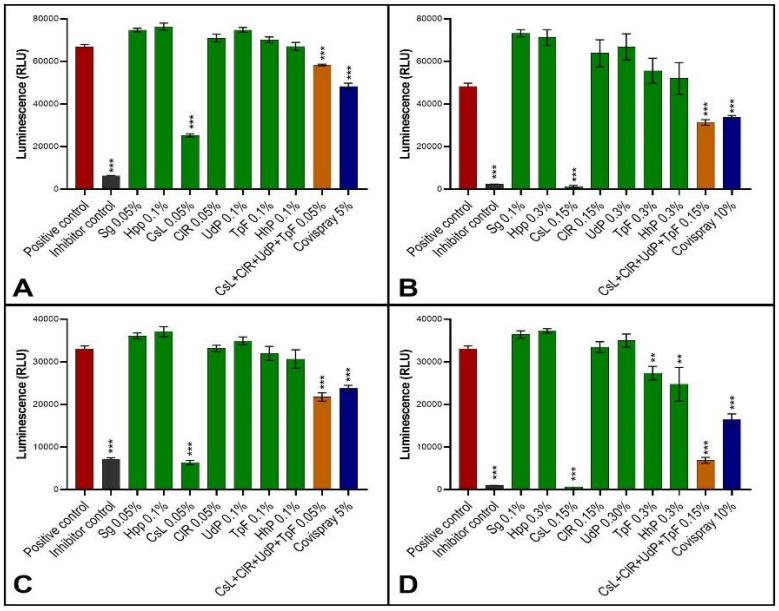


Figure 1 *: Mean (n=4) inhibition of luminescence (RLU) due to the binding of Sg or HpP (excipients); CsL, ClR, UdP, TpF individual plant polymers; association of these 4-polymers, and Covispray finished composition, at a lower (A, C) or slightly higher (B, D) concentrations with SARS-CoV2 spike S1 proteins (A, B) or RBD (C, D) proteins. Polymeric association called S1-cyanidins (CsL, ClR, UdP, TpF) are derived from tannins rich whole plant extracts. Comparison of the luminescence with one-way ANOVA followed by Sidak post'hoc. Confidence intervals 95%. *p<0.05 **p<0.01 ***p<0.001*

(3) Polymeric binding with Covid-19 specific cytokines

The Covid-19 pathology mobilizes a wide variety of immunological biomolecules, particularly the pro-inflammatory cytokines such as IL-6, TNF-α, IL-10, IL-13, & GM-CSF. The binding potential of Covispray polymers with these key cytokines was evaluated using ELISA tests at fixed polymeric concentration of 0.10% in 5.0% glycerol aqueous solution. The finished Covispray liquid formulation was also tested at 5.0% concentration. Test products were incubated with human purified recombinant cytokine (Invitrogen, 400 pg/ml) for 5 minutes in PBS. The remaining free and available recombinant cytokine is measured using Specific ELISA kit (Invitrogen, Human IL kits) according to the manufacturer's instructions. Recombinant cytokines without polymers were used as negative controls. The Optic Density (OD) is measured at 450 nm using an ELISA plate reader (luminometer-Envision, PerkinElmer).

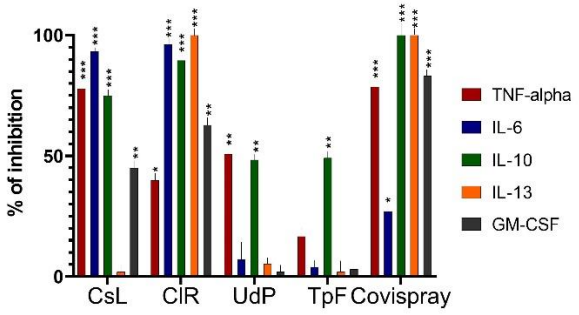


Figure 2 *: Covispray® individual polymer (0.10%) and 5% finished formulation mean binding (n=18 each) with Covid-19 specific TNF-α, IL-6, IL-10, IL-13 and GM-CSF pro-inflammatory cytokines using ELISA technic. ClR polymer blocks nearly 90% of TNF-α, IL-6 and IL-13 cytokines but has no effect on Covid-19 S1 and RBD proteins. One-way ANOVA followed by Dunnett's post'hoc. Confidence intervals 95%.*
**p<0.05 **p<0.01 ***p<0.001*

Results show that the ClR polymer was the most active in blocking nearly 90% IL-6, IL-10 and IL-13 and about 60% GM-CSF while CsL neutralized >75% TNF-α as well as IL-6 and IL-10 (all p<0.001 v/s controls). At 5% concentration, the final Covispray composition neutralizes nearly 30% IL-6 (p<0.05 v/s controls), 80% TNF-α and GM-CSF (p<0.001) and above 90% IL-10 and IL-13 (p<0.001). Therefore, it can be considered that 100% Covispray should neutralize most of the key pro-inflammatory cytokines.

Complete reports for above studies are available.

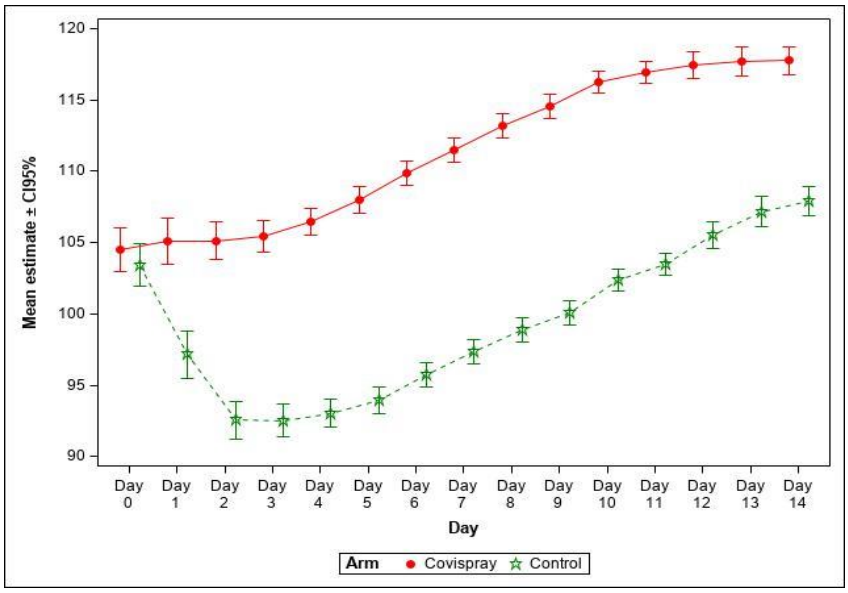
CLINICAL EFFICACY

Clinical efficacy (Vitrobio's product code: PIRDAL; Vitrobio product name: Covispray)
Protocol N°: PIR/OBS/2020; Version No.: 4.0, Dated: 03/11/2020
Test products: PIRDAL (Covispray) 15-ml nasal spray, 2-3 sprays in each nostril, 4-5 times per day. Ethical committee suggested that all patients should be allowed to take any standard symptomatic treatment (ex. aspirin, anti-inflammatory, anti-diarrhoea drugs, ayurvedic or natural treatments but no anti-viral or other drugs which may affect study outcome) prescribed by the patient's medical consultant throughout the study.
Control product: Only symptomatic treatments like that of the test product group.
Study title: A randomized, multicentric, single-blind, observational study to evaluate efficacy & safety of PIRDAL nasal spray in preventing symptomatic manifestation of disease in Covid-19 proven cases.
Place of study: India (Mumbai).
Trial registration: Clinical trials registry India (http://ctri.nic.in), CTRI n°: CTRI/2020/11/029388, Registered on: 26/11/2020
Investigator: MUDRA CLINICARE, Koparkhairane, Navi Mumbai-400709, India
Study authorisations: Indian health authorities (closely monitored)
Ethical committee: Altezza Institutuional Ethics Committe, Shree Ashirwad Hospital, Dombivli, Maharshtra, India (approved on 14/11/2020)
Duration of treatment: 14 consecutive days
Start / End date: November 2020 – January 2021
Patients: Randomized, included just after + RT-PCR test, confined at home with or without family members, closely monitored daily by government medical professionals, hospitalisation in case of any life-threatening symptoms due to ethical reasons.
N° of patients: Planned 200; Enrolled 213; Completed: 98 in Control standard treatment (ST) group and 102 in Covispray (ST) group.
Drop-outs: Total 13. ST group 8; ST+TP group 5.
Reason for drop-out: Low O2 saturation or sudden aggravation of symptoms.
Independent auditing company: RAJ CONSULTANCY, Plot 61, Sector 10, Kamothe, Navi Mumbai
Independent statistical & result analyses: Soladis Statistics, 6-8 rue Bellecombe, 69006 LYON – FRANCE
Primary and secondary endpoints: The primary endpoints were based on the key Covid-19 initial symptoms and included (1) change in the intensity of respiratory symptoms employing widely accepted and validated 19-parameter Leicester Cough Questionnaire (LCQ), Visual Analogue Scales (VAS) and cough-specific quality of life questionnaire (CQLQ) which are the most widely used health status questionnaires for adults with chronic cough. The results were compared to baseline at each time point, for each group, from the daily questionnaire filled by each patient during the 14-day trial period. Other general symptom scoring included fever, loss of taste, change in smell, headache, body aches, body pain, sensation of weakness, sore throat, eye irritation, day & night cough frequency, incidence of nausea, blood oxygen saturation, day & night cough frequency, nausea as well as all adverse events in both groups.

Preliminary results:

1. **Primary endpoints -17 cough parameter Leicester Cough Questionnaire (LCQ)**

LCQ, contains 19 specific cough related questions including 8 physical items, 7 psychological items and 4 social items. Each item is rated daily by the patient according to the frequency of occurrence with a score ranging from 1 to 7 where 1 represents severe cough symptoms and 7 the least.



LCQ result: The mean total rating of 17 individual scores in the study varied between 92,5 to 117,7 (mean for 17 parameters between 5.44 to 6.92) during the course of the trial. At randomisation (day 0) the mean of total LCQ scores was 103.4 ± 0.77 in Controls (n=106) and 104.5 ± 0.77 (n=107) in the Covispray test product group (NS on day 0). This shows that the mean of respiratory – cough related clinical symptoms were closely identical in the two groups and the population distribution was homogenous.

Control ST group: On day 1, nearly 24h after the start of treatment, the mean coughing symptoms sharply aggravated in the control group (mean score reduction of -6.3) which continued worsening in severity on day 2 (mean score reduction -10.9 compared to day 0 score in control group). The severity of cough remained identical or worsened very slightly between the days 3-5 but the symptoms improved thereafter, slowly and progressively, up to the end of the study.

In the Covispray treated group, there was no increase in LCQ mean scores from the 1st day of treatment up to day 3. The symptom severity remained stable during these days (mean score 104.5 ± 0.77on day 0 and 105.4 on the day 3 ± 0.57). From the day 4 onwards, the mean cough symptom intensity started improving, slowly and progressively, up to the day 14 (111.5± 0.42 on day 7; 116.2± 0.38 on day 10; and 117.8± 0.50 on day 14). Due to the stabilisation of clinical symptoms in the test product group right after the 1st treatment in the Covispray treated group during the 1st 3 days, the difference with the control ST group was highly statistically significant v/s ST ($p<0.001$) during the entire study period.

We hypothesize that the stabilisation of the LCQ symptoms observed in this clinical trial right after the 1st product application in ST+TP group is probably related to instant and continuous cleaning of free virus particles and pro-inflammatory cytokines from the nasal surface. Cleaning the nasal surface, stopping new virus infection, and minimizing nasal mucosa inflammatory cytokines, on the day 1 should create a favourable environment for nasal mucosa repair. This mode of action stabilizes disease pathogenesis.

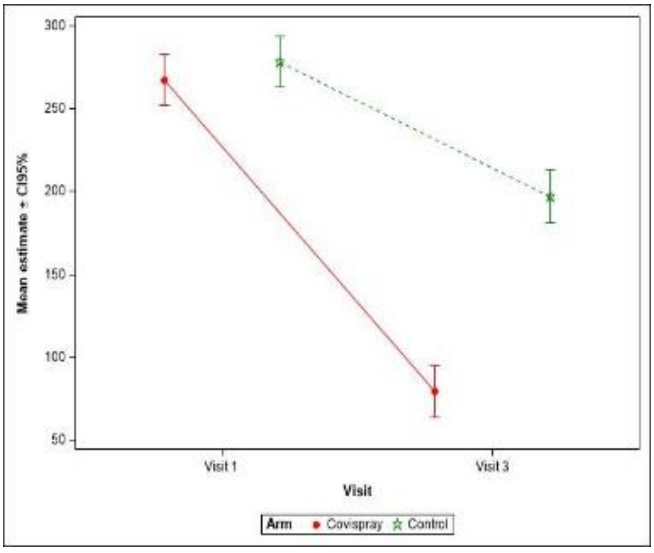
Being an exclusively topically acting device, the product cannot affect the growth of virus growing intracellularly. These viruses continue multiplying and pouring on the nasal mucosa. The newly generated virus particles continue releasing on the nasal surface where they are osmotically attracted towards the film and neutralized. This is probably the reason why the disease symptoms do not stop immediately but reduce progressively.

Reducing inflammation, virus load, and enhancing nasal mucosa natural defence capacity should in turn reduce immune load and allow immune system to better defend the infection. This is probably the reason why fewer number of patients (5/102 in ST+TP v/s 8/98 in ST) developed severe clinical signs and were hospitalized in the Covispray group.

As virus was already present in the body, it will continue reaching the nasal mucosa and infecting new cells, during a certain period, up to the time al the virus particles are not neutralized or cleaned from the nasal surface. Therefore, we cannot hope that Covispray should cure the disease or stop all the disease symptoms immediately, but it should only stabilize the disease progression immediately after the 1st application.

2. Mean Visual Analogue Score (VAS)

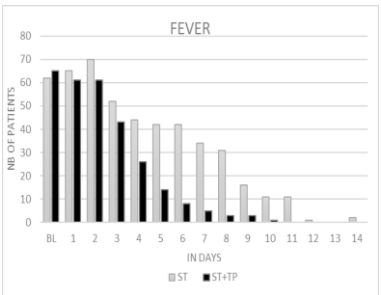
The VAS employs a linear scoring method that has a straight line with calibration of 0 to 10 cm; 0 indicates asymptomatic, and 10 represents the most serious. The scores are noted by the patients. In this study, LCQ, being highly precise and measured daily for 17 cough related parameters, the VAS has less importance. In this study summary, VAS is calculated only for the Visit 1 and 3.

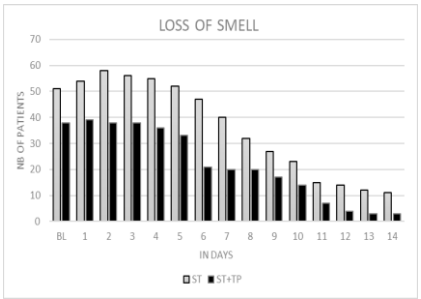


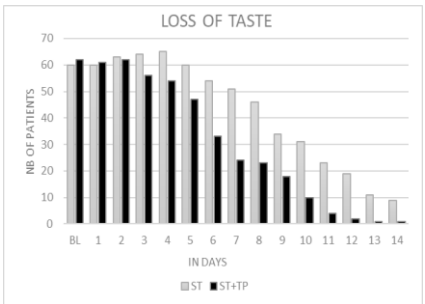
VAS Outcome: At the start of the study, the mean total VAS scores in the control ST and the Covispray (ST+TP) groups were 267.4 ± 7.80 in the ST and 278.4 ± 7.84 in the ST+TP groups (- 10.7 in the ST+TP group). The difference between the two groups is not statistically significant at the start ($p<0.320$, NS). As the cough symptoms started reducing in both the groups from day 4 to 6 and improved considerably on the day 14 (Visit 3), its logical that the mean total VAS scores also reduced in both the groups towards the end of the study. At visit 3, the mean score in the ST group was 197.0 ± 8.15 but was only 79.40 ± 7.99 in the ST+TP group with a difference of 117.6 ± 11.41 in favour of Covispray group. This difference between the two groups is highly statistically significant ($p<0.001$, S).

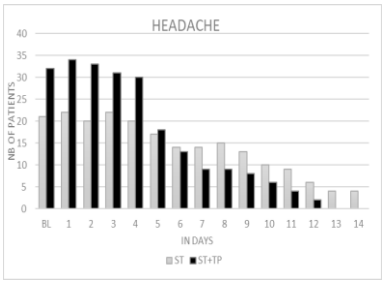
These results correspond to the LCQ results where a strong improvement in respiratory cough symptoms was noticed during the 1st 3-4 days in the ST+TP group and the same is confirmed in VAS parameter. These results reflect that Covispray treatment significantly supress cough related parameters in a slow and progressive fashion.

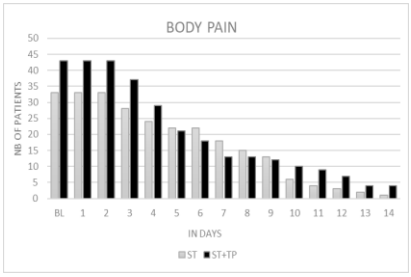
Other key parameters: *Effect on fever, loss of smell, loss of teste, headache, body pain, and sore throat recorded daily by each patient. (n=98 in ST and 102 in ST+TP) on each day in each group. Results are expressed as number of patients affected at each time point. BL = baseline data on day 0.*











Effect on fever: The number of patients having fever at BL and on the day 1 was nearly identical in both groups (about 65% patients). From the day 2 onwards, fever progressively decreased in both groups, but the reduction was much faster in the ST+TP group with only 43/102 patients had fever on day 3, 26/102 on day 4, 14/102 on day 5, only 1/102 on day 10 and none from the day 11 onwards. In control group, 34/98 patients had fever on day 7 but the incidence reduced slowly with 11/98 patients on day 10 and only 2/98 on day 14. These results show that cleaning the contaminants from the nasal mucosa at the start of the disease dramatically reduces the incidence of fever.

Loss of sense of taste: Is an important clinical sign in Covid-19 infected patients. The Covispray treatment had no effect on restoring loss of taste during the 1st 2 days of treatment compared to the baseline values but in this group a progressive recovery of tase was observed, particularly for the day 5 onwards. Only 25% patients complained of taste loss on day 7, 10% on day 10, and only 1% on day 14. In the control group, the loss of taste remained unchanged during the 1st 5 days (60/98 between days 0 to 5) with a progressive recovery of taste from the day 6 onwards (30/98 on day 10 & 9/98 on day 14). The recovery of taste loss is faster in Covispray treated group, which is concomitant with the improvements in LCQ, VAS, and fever observed in this group.

Effect on loss of smell: Only 38/102 patients in the ST+TP group complained of loss of sense of smell compared to 51/98 in the control ST group at the time of recruitment. This shows that less Covid-19 + patients suffer less from loss of smell then the loss of taste. Smell sensation started improving in both groups only after day 5 but the reduction was slightly better in the ST+TP group.

Effect on headache: Only 21 patients in the ST group complained of headache compared to 32 in the ST+TP group at the start of the study. In both the groups, the incidence of headache started decreasing only after the day 4 but the reduction in the ST+TP group was much faster compared to the ST group patients from the day 5. Thereafter, the reduction in the ST+TP group was fast and no patient complaint about headache from day 12. In the ST group, the reduction was progressive and still 4/21 patients continued having headache on the day 14. These results show that the reduction in LCQ and VAS parameters observed in the ST+TP group during the 1st 4 days of treatment equally affect other health parameters simultaneously.

Effect on body aches & pain: The number of patients having body aches and pain was nearly 40% higher in the ST+TP group at baseline compared to the ST group, with no change in this parameter up to the day 3 in both groups. The incidence started decreasing in both the groups from the 3rd day of treatment, but the improvement looks slightly better in the ST+TP group. It is concluded that there is no significant difference between the two groups with respect to improvement on body aches and pain. This may have been related to the fact that virus continue residing inside the body and generating inflammation which is not much affected by cleaning the contaminants only from the nasal surface.

Effect on sore throat: At BL, the incidence of sore throat was observed in 20/98 patients in the ST group and in 28/ 102 patients in the ST+TP group. There was no change in this parameter in both groups up to the day 3 but from the day 4, there was a drastic reduction of sore throat in the ST+TP group with only 7/28 patients with sore throat on day 6, 2 on day 10 and 0 on day 12 v/s 14/20 patients on day 6, 10/20 on day 10, 4/20 on day 12 in the ST group. No patient complaint about sore throat in both groups on day 14.

Other parameters: Feeling weak – strong decrease in ST+TP group (n=27 patients) from day 3 onwards, 1 on day 14 v/s ST group (n=25) with 7 patients feeling weak on day 14.

Eye irritation: n=34 in ST+TP and 27 in ST. Identical results in both groups. Covispray had on effect on reducing eye irritation.

Day cough frequency: Day time cough was more prominent than night cough in both groups. At BL, 54/98 patients in the ST group and 42/102 (difference of 12+) in ST+TP group had cough during the daytime. The number of patients with this complaint continued increasing in the ST groups up to day 9 (53/98) while in the ST+TP group, this increase was only up to day 5 (47/102) but started reducing rapidly from day 6 (38/102) and only 15/102 patients on day 9 (compared to 53/98 in ST). No patient had day cough in ST+TP group from day 13 but in the ST group, still there were 21 patients having cough on day 14. These results show that the test product take 5-6 days to supress strongly daytime cough and this suppression is very strong compared to the ST group.

Night cough frequency: About 35% patents (n=37 in ST and 35 in ST+TP) had night coughing at BL. Identical to the observations of day coughing, the number of patients with night cough continued increasing in both groups during the 1st 8 days in the ST group but only during the 1st 4 days in ST+TP group. On day 7, 43 patients in ST but only 28 in the TP group had coughing during night. On day 10, there were 30 patients with this symptom in ST compared to 8 in ST+TP and on day 14, there were no cases of night cough in ST+TP group while there were still 13 patients in the ST group. These results further confirm the finding that Covispray minimize the duration of the disease symptoms compared to all other standard treatments used nearly identically in both groups.

Effect on nausea: 4/98 patients in ST and 11/102 in ST+TP had nausea at BL. In both groups the number continued decreasing (0 on day 10) but the reduction was much rapid in ST+TP group.

These were no significant difference between the two groups for other parameters including blood O2 saturation level, respiratory rate, blood pressure, heart rate, and pulse rate.

Adverse events: There was no moderate or severe adverse event recorded in both groups during the study. 13 patients (8 in ST and 5 in ST+TP) were dropped out because their blood O2 level decreased or some of the Covid-19 clinical symptom deteriorated in these patients. There was no mild adverse event noticed in any of the ST group patient but 42/102 patients in the Covispray (ST+TP) group had mild adverse effects related to the application of the test product. The main events were (1) nasal irritation or nasal discharge, usually during the 1st few minutes just after the product application, and (2) watering of eye after product use in as many as 42/102 patients. These effects are considered related to the product because nasal mucosa osmosis starts immediately after product application which attracts hypotonic liquid from the nasal surface. This sudden reduction of intracellular liquid is felt as mild to moderate irritation which may even lead to eye discharge. The cellular liquid loss is immediately (within 1 min) replaced by circulating liquid which stops further feeling of irritation.

As this event is related to the mode of action of Covispray hypertonic filmogen osmotic solution, and as it has no residual adverse consequences, these effects are considered not harmful to the patient.

One case of nausea and one of vomiting are isolated cases and are common in Covid-19 symptomatic patients. Therefore, they are not considered as related to the product.

Conclusion: The test product acts mechanically just by inducing a strong osmotic liquid exudation from the nasal mucosa and by cleaning the nasal surface of all the free-floating contaminants. As newly generated virus particles, at least from the nasal mucosa, are continuously attracted towards the film and trapped by polymers, new virus growth in the nasal mucosa should reduce and consequently the destruction of nasal mucosa barrier should be minimized. Reducing the concentration of pro-inflammatory cytokines from the nasal mucosa surface should help reduce inflammation and stimulate reconstitution of nasal mucosa. The nasal surface cleaning effect starts within a few minutes after product application and lasts 4-6h after each product application which may be the main reason behind immediate stabilization of clinical manifestation of the disease observed in this study. As the intracellular virus continues growing and pouring on the nasal surface and probably also a fraction of circulating virus reaching the nasal host cells for growth, the infection in the Covispray treated group stabilizes without progression but remains moderate or mild up to the time the body's immune system continues fighting against the infection.

Covispray is not a curative treatment against Covid-19 but is an excellent and safe topical treatment to supress and to stabilize the disease progression nearly instantly. In the absence of any specific treatment against this disease, Covispray can be used in the early phase to minimize the occurrence of severe cases of respiratory distress. It can also be used as a nasal cleaning device in case of possibility of contact with an infected person or as a precautionary measure.

Other information:

Type of product: A Class I Medical Device in EU. Made in France. (Class IIa in 2021, all dossiers available). **Justification to classify the product as a Class I Medical Device**: As per the European directive 2001/83/EC. **Stability**: Validated 24-months.

Note: The name Covispray cannot be used in European countries. Being the most important current health concern, we recommend taking advice of the local regulatory authorities before launching the product in any country. Complete dossiers for each claim and mode of action are available. This technology is protected by 3 international patents.

Further information: contact VITROBIO, France. *rs@vitrobio.com*

VITROBIO France: Recipient of European PAEXA & French Academy of Science Awards

VITROBIO, ZAC de Lavaur 63500 Issoire, France.