

ISSN: 2641-1911 Archives in Neurology & Neuroscience

Research Article

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Proven Novel Method to Curtail Covid-19 Pandemic, Long Covid, any Future Viral Pandemics, Including Influenza, Due to RNA/DNA Viruses Through Inactivation of their Naked RNA/DNA, and Hospital Acquired [Nosocomial] Infections

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Abstract

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The main object of this research oriented experimental investigation is to evaluate the methods to totally inactivate the SARS-COV-2 and its newly developed variants, including their genetic material [naked RNA/DNA]. The viral naked RNA is referred to as RNA outside the body or integral structure of the virus. The United States Patent # 11,643,641 B2 was issued on May 9th, 2023, with a title "Prevention of Viral Transmission by Naked Genetic Material". The research pertaining to this patent was initiated in the years 2020 and 2021 at which time the majority of the Corona Viral variants that were causing Covid-19 infection and Pandemic were: Alpha [B.1.1.7]; Beta [B.1.351]; and Delta [B.1.617.2] and several of their subvariants belonging to the SARS-CoV-2 viral lineage. Most of the experiments apparently were directed towards inactivating the above SARS-COV-2 variants. Due to continuous mutations encountered in the genome of the variants of the original SARS-COV-2 Corona Virus, several new variants were developed towards the end of the year 2021. Out of all the prior variants, Delta has more transmission rate as well as the dominant version of causing Covid-19. By mid-December of 2021 and early 2022, the new variant Omicron emerged onto the scene. According to several experts including CDC most of the original variants including Delta are now non-existent in the year 2023. The predominant variants and subvariants of the omicron became the major contributing factors to cause Covid-19 during the year 2023. Since our patent application was submitted on Feb 25, 2022, [Patent was issued on May 9, 2023], in all probability our field experiments must not have involved any attempts to check for the efficiency of our Novel procedure to inactivate the newly evolved Omicron and its variants and subvariants. Thus, this R&D project is specifically undertaken to check the efficacy of the Patented procedure outlined in the US Patent # 11,643,641 B2 on inactivating the Omicron variants evolved during the year 2023, [although earlier we have proved that Delta variants were inactivated], in an attempt to use it as an effective tool confidently to control and curtail the current and unforeseen future Pandemics. The laboratory studies conducted, using the saliva samples of the RT-PCR test positive SARS-CoV-2 infected persons, distinctly proved that the procedures outlined in US patent # 11,643,641 B2 are valid and extremely effective in inactivating the Omicron virus and its viral naked RNA, including denaturation of the viral nucleocapsid, spike, membrane, and envelope proteins. We have also confirmed that mechanical microbial vectors [which can also cause hospital acquired [Nosocomial] infections, which help to spread the viral particles including their naked RNA or DNA can also be totally inactivated using the novel Patented procedure outlined in the US patent # 11,643,641 B2. The details of which are outlined with explicit details in this research article.



The detailed sophisticated techniques were developed, for the first time, to study the effect of the naked viral DNA belonging to bacteriophage specific to bacterium Streptococcus thermophilus, to infect the host, propagate, and lyse the cells, without the aid of appendices such as head and tail structures, proving viral naked DNA by itself can also cause infection. Detailed pictorial illustrations are presented to show the mechanism of Covid-19 infection by the SARS-CoV-2 virus, and its naked RNA, with the aid of mechanical microbial vectors. Experimental proof is presented to show that the novel patented procedure [US Patent # 11,643,641 B2] can be effectively applied to control not only Covid-19 pandemic but also any future pandemics due to enveloped or non-enveloped RNA and/or DNA viruses. The patented procedure works irrespective of number of mutations involved in the mutants and sub mutants belonging to the lineage of the SARS-COV-2 virus, and for that matter any viral lineage, including but not limited to influenza virus etc.

Key words: Naked RNA; Naked DNA; Long Covid; SARS-CoV-2; RNASE; DNASE; Covid-19; Delta and Omicron Variants; Hospital Acquired infections; Viral and Microbial Pandemics; Nosocomial Infections

Introduction

SARS-COV-2 first emerged in Wuhan province in China in November 2019. By March 2020, it did spread over 220 countries at the fastest pace. World Health Organization has designated the disease caused by SARS-CoV-2 [Severe Acute Respiratory Syndrome- Coronavirus-2] as Covid-19 [Coronaviral Diseaseemerged in 2019] [1,2]. It was declared as a Pandemic and over 700 million people were infected with a total death of seven million people. Technically the Pandemic is not contained yet. The major concern in 2023 is the Covid-19 disease caused by variants of the Omicron, especially the new variant called EG.5 [nicknamed "Eris"] and another strain called BA.2.86 [nicknamed "Pirola"]. These two variants of the Omicron emerged only in the middle or later part of 2023 [3]. Before proceeding further into this research article, I would like to clarify the meaning and definitions of the following terms used in this article to avoid confusion to the readers: Lineage; Sub Lineage; Variants; Sub Variant; Mutation; Recombinants; Pango Lineage System; and Long Covid etc.

A Lineage is a group of closely related viruses with a common ancestor. The original neo-SARS-CoV-2 evolved in 2019 serially mutated over 3 years to generate Alpha, Beta, Gamma, Delta, and Omicron variants etc. Yet all of these variants belong to the lineage of SARS-CoV-2 [common ancestor] and all of them cause Covid-19 disease in humans.

A Sub Lineage is defined as a lineage being a direct descendent of Parent lineage. For example, the Omicron BA.2.75 is a sub lineage of Omicron BA.2.

Variant: Over a period of time, the virus may start to differ slightly from its parent strain, in terms of its molecular genetic sequence. Although, it is termed as a mutation, such viruses with new mutations can also be called variants. As an example, Alpha, Beta, Gamma, Delta, and Omicron are the variants of the novel SARS-CoV-2 virus, which emerged in 2019. The World Health Organization [WHO] and the SARS-COV-2 interagency group [SIG] has categorized variants into Variant of Interest [VOI], Variant of Concern [VOC], Variant of High Consequences [VOHC], and Variant Being Monitored [VBM] on the basis of the severity of the infection, resistance to the medications including vaccines, rate of transmissibility etc., which may require public health action. The Delta variant [B.1.617.2] can be categorized as VOHC. However, currently as of September 28, 2023, the dominant variants of Omicron, nationwide are EG.5 with 24.5% cases, followed by FL.1.5.1 with 13.7% cases and XBB.1.16 with 10.2% cases.

Mutation: The term mutation refers to a single change in the viral genetic code. Mutations happen frequently, but they are only recognized when the characteristics of the mutated virus change significantly.

Sub Variant: A Variant with an additional mutation is called Sub Variant [Mutation on top of the Mutation]. Omicron [Variant of Delta] BA.1 additionally mutated in the genome which was found to be slightly more infectious and was designated as a sub variant with an identification of Omicron BA.2.

Long Covid: Each Covid -19 infection carries the risk of Long Covid, [although tests prove the patient is negative for Covid-19], which is a syndrome with ongoing Covid symptoms, which can last for weeks or months or a year after infection. According to the published information, over 23 million Americans have long Covid and perhaps worldwide over 100 million people may be suffering from Long Covid.

Recombinants: When two different variants or sub variants enter into a human cell simultaneously and exchange their genes, the newly developed virus is called a recombinant. A classic example is the original Omicron BA.1 and BA.3 [a sub variant of BA.1] when interact together, several recombinant sub variants evolve with slightly different infective patterns. Thus, BA.4, BA.5 are essentially the mixtures of BA.1 and BA.3 with additional mutations. Some of the recombinants pose a real threat to public health, especially when two genetically unrelated viral genomes combine and exchange virulent genetic determinants. This is especially true when both animal and human viruses interact to generate a new recombinant. Perhaps SARS-CoV-2 could have been a recombinant evolved due to exchange of both human and animal viral genomes, which shook up the world causing a world-wide Covid-19 Pandemic.

What criteria is followed to name the variants and sub variants for identification purpose?

Pango Lineage System: It is one form of nomenclature to identify the lineage. It is just like constructing a family tree. The lineages are named using an alphabetical prefix [such as B or BA] and numerical suffix [such as ".1" or "0.1.1.5"]. When a new lineage or variant is discovered or defined, Pango system assigns an additional number to the name of the parent lineage [such as BA.2.75 is a sub lineage of BA.2]. As more mutations take place in the virus, the Pango Lineage names get too lengthy. The lineages with lengthy names shall be given alphabetical aliases and numbering continues. For example, "BA" stands for "B.1.1.529" and thus BA.2 is the same as "B.1.1.529.2". I want to bring this to the attention of the readers, to eliminate further confusion on the nomenclature and designation of the variants and the sub variants of the viral lineages.

In the year 2020 the death rate suddenly increased due to Covid-19 infection. Despite all the attempts to curtail the spread of Covid-19, the rate of infection and death toll significantly increased during 2020, 2021, 2022, and part of 2023 totaling 700 million infections with a confirmed deaths of over seven million people around the world. Although, several vaccines and Boosters have been developed and administered, neither the infection rate nor the death rate reduced significantly to the point of curbing the Covid-19 [1,2,4]. Even the pharmaceutical drugs namely Remdesivir and Paxlovid did not work efficiently to protect the humanity. Ultimately it was declared that there is no magic bullet or a single therapy to treat Covid-19 infection [1,4]. Until now, there is no single vaccine that can confer immunity for a long period of time, due to continuous mutation of the SARS-COV-2 lineage resulting in Variants and Sub Variants with different antigenicity [5,6,7,8].

So far most of the attempts were aimed at producing antibodies against the Coronaviral spike proteins only. Unfortunately, since there was no proof reading while the SARS-CoV-2 was being produced within the cell, the rate of mutation was significantly high [2,9]. It was also discovered that the recent Omicron variants are getting insensitive to the vaccine activated antibodies thus overriding the vaccines. On top of it, the antigenically altered spike proteins of the Omicron variants and sub variants, due to uncontrolled mutations, have greater affinity to attach to the ACE-2 receptors sites, more than the previous variants including Delta, thus improving the infectivity rate with significantly greater rate of transmission. Consequently, it is getting harder and harder to totally curb the infections due to SARS-CoV-2 Lineage and their Sub Lineage. For example, initially the novel SARS-CoV-2 was highly virulent, and later the variant Alpha was more dominant and more virulent than its parent, in the later part of the year 2020. Further mutation of Alpha resulted in Delta variant, which was proven more lethal than the earlier novel SARS-CoV-2 and Alpha, during the years 2021 and 2022. As of the year 2023 the only variant and sub variants of Omicron are persisting and are responsible for Covid-19 infection [3]. The earlier variants are not in existence. Although the Omicron variant appeared in December 2021 and early 2022, its major impact is being felt in the later part of the year 2022 and rest of the year 2023. Although the death rate due to the Omicron variants infection [Covid-19] is low in comparison to Delta, its transmission rate is significantly higher [9].

Considering the above chronological events in terms of the progression of Covid-19 infection causing lineage and sub lineage

of SARS-COV-2 virus, the scientific community has to look into alternative ways of curbing the Covid-19 pandemic in addition to the development of vaccines and the pharmaceutical drugs.

As early as 2020, as soon as pandemic started, we undertook a research project on preventing or treating Covid-19 disease using biological therapeutic approach [1]. Sine the Covid-19 infection involves excess cytokine storm and a subsequent thrombosis resulting in death, due to respiratory distress and multi-organ failures, our approach was to simmer the cytokine storm through proper immunomodulation [1,2,9,10]. One of the factors we considered to induce proper immunomodulation was to alter the composition of the microbiota and microbiome using multiple mixed strain probiotics and their immunomodulins [11,12]. We have discovered that probiotics alone without their growth end products [immunomodulins] could not induce proper immunomodulation and thus could not control Covid-19 infection [1]. Four different patented preparations were developed using probiotics along with their immunomodulins. They are as follows: Nasal Purge, Mouth Wash, Nasal Inhaler [smelling salt], and tablets made using liposomal preparation for oral ingestion. Their composition is outlined in the US Patent # 11, 077,052 B1 [1], and other subsequent publications [2,9]. Later, over a period of time, we have discovered that the Covid-19 pandemic was not being contained despite all the attempts made by several organizations including pharmaceutical companies, vaccine producers, CDC, and WHO [13,14]. At this stage in the year 2021 we undertook another project, in an attempt to inactivate the SARS-CoV-2 and its variants permanently, outside the human body in the environment. We have suspected that there is something drastically different about this Covid-19 infection [13]. According to CDC, and other investigations, SARS-CoV-2 and its viral variants should fall apart in 3 days to one week while they are outside the human body [4]. It was determined on the basis of the laboratory testing and evaluation. However, they did not pay any attention to the live viral genetic materialnaked RNA. Although it is devoid of spike protein, nucleocapsid and membranes, it can still inflict infection, if it gets access to the human body [13]. We have proved using a DNA bacterial virus that a naked DNA bacteriophage can infect bacteria, even if it is devoid of the head and tail, protective structures and appendices required to inject the DNA into the bacterial cells [4]. The test protocol and results are presented in the research article.

All along we have suspected that the Naked RNA of the SARS-CoV-2 virus and its variants belonging to the same Lineage can still cause Covid-19 infection, even if they are devoid of Spike and other proteins and their supportive membrane [13]. A series of experiments were conducted to prove this hypothesis to offer a possible explanation why the Covid-19 Pandemic could not be contained despite the use of multiple vaccines, medications, and the precautionary measures including lock downs, observing social distancing, wearing masks etc. etc. We have also suspected that the Long-covid, which has affected over 100 million people around the world could also be due to the SARS-CoV-2 viral naked RNA. The Long-Covid symptoms are lingering for few months to an year [after the Covid-19 disease was cured], thus draining the economy due to shortage of labor, absenteeism, and excessive medical costs. Recently [October 24, 2023] a popular article, authored by Mr. Will Stone, appeared on the internet with the following title, "NPR: Long-Covid brain fog may originate in a surprising place, say scientists", which can be read in NPR: https:// Apple. News/. Aiuqmjm3jTvaug Ycu15Dd1g. According to the original article that appeared in the journal of CELL, the researchers at the University of Pennsylvania uncovered that Long-Covid patients exhibited significantly low levels of neurotransmitter serotonin in the blood [15]. In addition, the earlier investigations revealed that the stools of the Long Covid patients showed virus particles, long after the Covid-19 infection subsided [16,17,18,19,20]. The University of Pennsylvania researchers referred to the viral particles as viral reservoir [15]. In my opinion the viral reservoir constitutes perhaps intact viral particles, naked viral RNA, and the viral Spike and other SARS-CoV-2 virus associated proteins. Apparently, these viral particles in the GI tract may trigger the human immune system to produce specific interferons to fight the components of the viral reservoir. However, these interferons can cause severe inflammation which will reduce the absorption of the amino acid Tryptophan in the GI tract [15,21,22]. Tryptophan [which is a building block for the synthesis of several neurotransmitters] is required to generate serotonin, which carries messages between nerve cells of the brain and rest of the body. Serotonin is also the regulator of the Vagus nerve, which mediates communication between the body and the brain. Thus, the Pennsylvania researchers like their predecessors predicted that low levels of serotonin could be a causative factor for the Long-Covid symptoms, such as brain fog, loss of memory, loss of sleep, abnormal fatigue, depression, and delayed wound healing etc. [15,23]. According to the researchers serotonin depletion in Long-Covid can be attributed to viral RNA induced Type 1 Interferons.

Apparently, the Naked RNA of the SARS-CoV-2 virus can induce Long-Covid symptoms, in addition to causing Covid-19 infection. In our earlier research work, which was Patented, we have discovered that multiple mixed strain probiotics along with their immunomodulins can cure Covid-19 infection when administered as a nasal spurge and / or as liposomal tablet or liquid preparation administered through the oral route [1]. Now, based on the new research findings, it can be interpreted that specific probiotics and their immunomodulins can counteract the corona viral Naked RNA in the Gastrointestinal tract [13,24,25,26]. It explains why only some people exhibit Long -Covid symptoms but not all others who were also infected with SARS-CoV -2 virus with resulting Covid -19. It goes on to prove that the composition of the intestinal microbiota and microbiome plays a significant role in the prevention or treatment of Covid-19 and Long-Covid [1,2,9.27]. According to the University of Pennsylvania researchers, coronaviral viral Naked RNA perhaps plays a significant role in the genesis of Long-Covid. In my professional opinion, the Naked RNA from the environment can also cause Covid-19 infection, if it gains entrance into the human cell. Furthermore, it can also induce Long-Covid symptoms by stimulating the immune system to produce specific interferons which can diminish the tryptophan absorption and thus cause depletion of the neurotransmitter serotonin. The fact that the University of Pennsylvania researchers and other

researchers discovered the presence of SARS-CoV -2 viral particles in the stools of the Covid-19 recovered patients with ongoing Long-Covid symptoms proves that perhaps even the viral Naked RNA can induce Covid-19 infection [15,28].

According to several other investigators both the Covid-19 positive and Long Covid patients discharge enormous amount of SARS-CoV-2 RNA in the feces for an extended length of time ranging from few weeks to 10 months and even longer [15,16,18,19]. Consequently, the naked RNA must be prevalent in the environment. Thus, it is essential that the viral naked RNA prevalent in the environment and mechanical vectors, which assist to carry such viral naked RNA to infect humans, should be totally destroyed or inactivated to curb the viral Pandemics. It is reported in the literature that the Long Covid patients excrete both Genomic RNA [g RNA] as well as sub genomic RNA [Sg RNA]. The fecal viral RNA was detected even though the patient did not have any viral RNA detected in the Oropharynx, and thus proving that the Long Covid associates with the upper and lower GI tract [16]. Thus, long Covid can be categorized as latent SARS-CoV-2 infection, and the person who has Long Covid is a carrier of Covid-19, yet with different symptoms than the typical Covid-19 symptoms associated with the pulmonary alveolar tissues. Although, the etiopathology of Long Covid is obscure, in my opinion, it is due to naked RNA, which can get into human cells perhaps through the endocytosis route rather than with the aid of Spike protein interaction with cellular membrane. In addition, coronaviral RNA is a potent inducer of antiviral innate immune signal provoking antiviral state by directing the expression of interleukins and proinflammatory cytokines. The viral RNA induced interleukin and proinflammatory cytokines will disrupt the absorption of the amino acid tryptophan [controlled by ACE receptor-2 receptor0, which is required for the production of neurotransmitter serotonin. Lack of serotonin induces symptoms of Long Covid manifested in the form of Depression, Brain fog, joint pains, headache etc. The naked RNA of the SARS-CoV-2 virus, since it is a positive sense single stranded RNA can serve the purposes: one as the viral mRNA which is then translated into viral gene products and second as a template to produce more RNA strands. Considering the pathophysiology of viral RNA induced Long Covid symptoms, I can hypothesize with the confidence that the SARS-CoV-2 naked RNA can infect humans with the assistance of the mechanical vectors by either direct penetration into the cytoplasm through a partially disrupted cell membrane or perhaps gain entrance into cell through endocytosis path along with the nutrients such as amino acids and peptides. Since the only vital infective part of SARS-CoV-2 virus is its RNA, as long as it gains entrance into the cell cytoplasm even without the aid of Spike protein, it can produce virions using the host machinery. Net effect, naked RNA can not only cause Covid-19 infection, but also can induce Long Covid symptoms through stimulation of the immune system to produce interleukins and proinflammatory cytokines., which will hinder the absorption of amino acid tryptophan by impairing the ACE-2 receptor functions.

In addition, the Coronavirus and its naked RNA can alter the composition of the intestinal microbiota, which controls the immune system, to favor the growth of pathogenic bacteria while suppressing the probiotic micro- organisms. In essence, both the SARS-CoV-2 virus as well as their viral naked RNA can cause dysbiosis in the GI tract to cause multitude of symptoms resembling long Covid. I can substantiate the hypothesis with a solid research back up [US Patent # 11,077,052] wherein the Covid-19 infection was prevented or treated successfully with the use of multiple mixed strain probiotics along with their immunomodulins can either nullify SARS-CoV-2 virus and also may inactivate the viral naked RNA through the derangement of genetic code.

The current research is undertaken to study and validate the novel procedures outlined in the US Patent # 11,643, 641 B2, regarding not only the ways and means to inactivate SARS-CoV-2 virus but also to inactivate their Naked RNA in the environment, to prevent or contain the Covid-19 Pandemic. The results of this research will have greater additional benefits to humanity in that even the seasonal influenza epidemic can also be controlled by inactivating the viral Naked RNA. Equally the Naked DNA of the pathogenic DNA viruses can also be inactivated to curtail any of the future Pandemics associated with the DNA viruses.

Materials and Methods

The following experiments were conducted to study the effect of the novel discovery outlined in the US patent # 11,643,641 B2 on the inactivation of the virus and its RNA [including naked RNA] of the SARS-COV-2 viral lineage including the latest Omicron and sub lineage which caused Covid-19, and DNA Virus [including naked DNA], to curb the current Pandemic and any other future Pandemics due to RNA/DNA viruses including the seasonal influenza.

1) The effect of novel patented system [US Patent # 11, 643,641 B2] on inactivation of Delta and prior SARS-COV-2

variants [Alpha and Beta], using the saliva samples from Covid-19 positive patients, confirmed by RT-PCR test.

2) Field experiments to study the effect of the procedures outlined in US patent # 11,643,641 B2 to control or curb Covid-19 during the Pandemic years 2021 and 2022.

3) The effect of the patented system on inactivating Omicron variants of SARS-COV-2 [surfaced in the year 2023] through the denaturation of the Omicron Nucleocapsid [N] and other Spike [S], Membrane [M], and envelope [E] proteins, along with deactivation of naked RNA through enzyme hydrolysis.

4) The effect of the patented system on inactivation of the DNA viruses [bacteriophage] and also their naked DNA confirmed using standard plaque assay and electron microscopy.

5) Experimental demonstration of the ability of naked DNA of the Streptococcus thermophilus bacteriophage to infect bacterial host and successfully produce virus particles.

EXPERIMENT-1: The effect of novel patented system [US patent #11,643,641 B2] on inactivation of Delta and prior SARS-CoV-2 variants [Alpha and Beta], using the saliva samples from Covid-19 positive patients, confirmed by RT-PCR test.

Saliva samples of Covid positive patients were collected in sterile test tubes. The samples included in the study were tested for SARS-CoV-2, using RT-PCR test. The following methodology was followed to study the inactivation of the virus outlined in the US patent #11,643,641 B2. Three solutions were prepared, and they are designated as 1, 2, and 3. The compositions of the solutions are outlined in (Tables 1, 2, and 3).

Table 1: The composition of solution 1, in conformity with the ranges outlined in US Patent #11,643,641 B2.

Number	Ingredient	% Concentration
1	Mild Dilute Detergent ^a	92.4
2	Polysorbate-60	0.75
3	Sodium Bi Carbonate	3.75
4	Calcium Chloride	0.1
5	Chlorine	120 PPM (Final conc.)
6	Hydrogen Peroxide	3.0 PPM (To arrive at final conc.)

a = Mild Detergent may contain denatured alcohol, alkyl Dimethylamine, Sodium Laureate Sulfate, Phenoxy Ethanol, and other flavor ingredients, which is diluted significantly with water to enable easy spraying.

Table 2: The composition of solution 2, in conformity with the ranges outlined in US Patent # 11,643,641 B2.

Number	Ingredient	% Concentration
1	Water	99.5
2	Catalase	0.005
3	Sodium Thio Sulfate	0.05
4	Sodium Propionate	0.5
5	Natamycin (Pimaricin)	0.0001

Table 3: The composition of solution 3, in conformity with the ranges outlined in US Patent # 11,643,641 B2.

Number	Ingredient	% Concentration
1	Water	98.8
2	Cellulase	0.01
3	Alpha-Amylase	0.005
4	Catalase	0.005
5	RNASE	0.002
6	DNASE	0.0001
7	Sodium Propionate	1
8	Natamycin (Pimaricin)	0.01
9	Sorbic Acid	0.15

The test procedure conducted on Covid-19 positive saliva samples to evaluate the effectiveness of the methodology outlined in US Patent # 11,643,641 B2 to inactivate the SARS-CoV-2 virus and its variants.

Step-1: For 10 ml. of the Covid-19 positive saliva sample, 0.1 ml of solution 1 [as outlined in Table-1] was added and the sample was held at room temperature for 30 minutes.

Step-2: To the above sample, 0.1 ml of solution 2 [as outlined in Table-2] was added and the sample was held at room temperature for 30 minutes.

Step-3: To the above sample, 0.1 ml of solution 3 [as outlined in Table-3] was added and held at room temperature for one hour.

The RT-PCR test was conducted on Saliva sample before and after the aforementioned treatment. Since during the later part of the year 2021 and early part of 2022 the predominant variants of SARS-COV-2 causing Covid-19 were Alpha, Beta and Delta, we have concluded that the viral etiological factors also belonged to these variants [in all probability not Omicron Variants]. After the completion of testing, the control and test samples were autoclaved and disposed-off undertaking the sanitary principles. Wherever the saliva samples have watery consistency [rather than thick with mucus], after each step only 1–5-minute incubation was sufficient to run the test for Covid-19. The results of the experiment are presented in the results and Discussion section.

EXPERIMENT-2: Field experiments to study the effect of procedures outlined in US Patent # 11,643,641 B2 to control or curb Covid-19 during the Pandemic years 2021 and 2022.

Case History: This is a field experiment with the following case history. A major construction company in the Rocky Mountain region experienced severe down time due to continuous Covid-19 infections of the employees. At that time [during the years 2021 and 2022], according to the rules and regulations of CDC and local health departments in the US, anybody who tests positive for Covid-19 must stay at home and should not come to work for at least 2 weeks. If they were still positive for Covid-19 [using RT-PCR test] they were not supposed to come to work until they were confirmed negative for Covid-19 by their physician. According to the Company Management, even though lock downs were mandatory,

the construction workers were deemed essential workers and were given clearance to continue to work even during the lock down period. Yet, according to their management, the majority of their construction workers were coming down with Covid-19 infection confirmed by RT-PCR test, even though they have implemented strict sanitation procedures advocated by CDC and local health department, such as maintaining social distance, wearing masks, and sanitizing the hands and surfaces etc. No matter how strict the management was in instituting the sanitary procedures to prevent Covid-19, they could not control the infection and consequently the company experienced severe down time due to sick employees. Surprisingly, even the vaccinated employees were getting reinfected with Covid-19 causing Corona Virus.

The company management concluded that there is something more to it, which is unknown, causing the continuous Covid-19 infections among the construction workers. Upon the request of the company, we have supplied them with the solutions prepared as per the procedure outlined in the patent application. The composition of the solutions was outlined in Experiment 1. They were asked to spray the solutions in a sequential order. The solutions were supplied at no cost since the patent was still pending and not approved in 2022. After they instituted the procedures and continued for a period of approximately 6 months, employee absenteeism came down significantly, monitored by the Covid-19 testing using RT-PCR test [to differentiate from Covid-19 Vs common cold]. In addition, management was asked to keep track of the rate of reinfection even in the vaccinated individuals, while they were using our test procedure. We have also supplied materials for them to experiment with even the six-step procedure outlined in the US Patent # 11,643,641 B2 to check its efficacy in comparison to the three -step procedure. The results and observations of these field trials are presented in the results section of this research article.

EXPERIMENT-3: The effect of the patented system on inactivating Omicron variants of SARS-COV-2 [surfaced in the year 2023] through denaturation of the Omicron Nucleocapsid [N], and other Spike [S], Membrane [M], and Envelope [E] proteins, along with deactivation of naked RNA through enzyme hydrolysis.

The Covid-19 positive saliva samples were collected from two individuals [Male and Female] in September and early October 2023 with following history, background, and symptoms.

CASE HISTORY [Male Subject]

Subject: Caucasian White Male

Age: 28 Years.

Marital status: Married

Health Status: Type-1 Diabetes

Medication: Insulin by pump delivery system.

Dose: 100 units/Day

Drug: Novolog.

Overall health: Excellent other than Type-1 Diabetes from the age of 10.

Prior History of Covid-19 infection: Covid positive in March 2020 [RT-PCR Test]

Vaccination History: Vaccinated in 2021 October [Pfizer]

Covid Infection after Vaccination: Covid Positive in September 2023

Supplements: does not take any Vitamin or Herbal supplements

Case History: travelled to Dubai on business in late September 2023. No signs of sickness in Dubai. Travelled back to States end of September [29th]. The approximate flying time was 23 hours with layovers.

Starting at Dubai: 3 hours wait at Dubai airport.

Dubai to Zurich [Switzerland] by Swiss Air. Aircraft is Jam packed. Layover at Zurich for 3 hours. Zurich to Chicago -10.30 hours flight by United Airlines. Chicago to Denver, it took 2.30 hours flight by United Airlines.

During the flight no cough or cold was felt. He felt more fluid at the bottom of the tongue and felt like spitting frequently. After landing in Chicago, he felt that his chest was heavy with slight discomfort in breathing with slight cough. Additionally, his body started paining and had difficulty in walking with slight dizziness. He Went home from Denver Airport by Cab-Friday [29th September]. Apparently, he picked up the viral infection [later confirmed Covid-19] either at the Airport or in the Aircraft cabin.

Saturday [30th September]: His chest pain increased with more cough and sore throat with a temperature of 102.1 F. He called the travel doctor in the morning. They arrived at 11 AM, ran the Covid -19 home test. Also took the samples to run

RT-PCR test. He called the author Dr. Reddy and talked about the symptoms that he had been experiencing. Dr. Reddy asked him to collect the saliva sample as much as he could in a vial and preserve it. He did as he was told.

Sunday [October 1st], his physician confirmed that RT-PCR test was positive for Covid-19 and put him on Paxlovid medication, [3 tablets in the morning and 3 in the evening along with 1000 mg of Tylenol to be taken every 6 hours] and was asked to drink lots of water. Chest pain, body aches, sore throat persisted all through

Sunday.

Monday [October 2nd], Fever and body pains came down slightly, but sore throat and cough persisted.

Tuesday [October 3rd], Fever came down and felt slightly normal. Body pains were slightly reduced, and the chest congestion was significantly reduced with persistent cough.

Wednesday [October 4th], Everything back to normal including body temperature with some tiredness.

Thursday [October 5th], the patient was back to normal, yet feeling tired. The saliva samples were treated using the novel test procedure outlined in Experiment 1 and tested for Omicron variants using antigen test as well as the RT-PCR test.

CASE HISTORY [Female Subject]

Subject: Caucasian White Female [Wife of the Covid positive patient who came back from overseas on September 29th], who did not travel to Dubai.

Age: 27 years

Health Status: Excellent [No diseases, No hypertension, no diabetes, and overall, extremely healthy]

Prior History of Covid-19: Positive in 2020 [RT-PCR Test]

Vaccination History: Vaccinated in 2021

Covid Infection after Vaccination: Positive for Covid on October 1st, 2023.

Supplements: Takes Vitamin C, Multivitamins on a daily basis for a long time.

Latest Case History: After her husband came back from Overseas on September 29th, 2023, and was confirmed Covid positive on September 30th, she contracted Covid -19 on October 1st, 2023, which was confirmed by antigen test.

Monday [October 2nd]: She had body pains, chest pain, back pain, and no cough. Took Tylenol, more Vitamin C and drank lots of water. The temperature was 100.8F. [Saliva samples were collected].

Tuesday [October 3rd]: Body pains and Chest pains have been slightly reduced. The temperature was 98 F.

Wednesday [October 4th]: She was back to normal with no pains.

Saliva samples were taken on Monday [October 2nd] for the microbiological, antigen test, and RT-PCR test to study the effect of the patented invention on inactivating the current variants and subvariants of Omicron belonging to SARS-COV-2 lineage.

In this instance, female subject did not have severe symptoms as her husband did, indicating that she did not have any Comorbid conditions like her husband proving that Omicron variants can induce severe Covid -19 infection in the subjects who have long term comorbid conditions. Apparently, the new Omicron strain of SARS-COV-2 virus has a high transmission rate.

The following experiments were conducted on Saliva samples obtained from the subjects

The solutions 1,2, 3 outlined in experiment-1 were prepared as per the range limitations and guidelines outlined in the US Patent #11,643,641 B2. The test protocol followed as per the procedures outlined in experiment 1. In addition, two different variables were introduced in solution 3. One is the same composition as outlined in experiment 1, and the second one is similar composition except RNASE and DNASE enzymes were eliminated to check for the effect of these enzymes in inactivating the Coronaviral naked RNA. After step-2, the samples were analyzed by using both the rapid Antigen test as well as the RT-PCR test and similarly, after Step-3 [using two different composition solutions i.e., with and without DNASE and RNASE enzymes].

The Saliva samples were analyzed for the presence of Omicron Variant Antigens by using the rapid antigen test, and the RNA by using the RT-PCR test

In addition, microbiological tests were also conducted in the saliva samples, before [control] and after the treatment [Test]. The microbiological tests conducted were as follows: Yeast and Molds; Enterococci; and Coliforms. The yeast and Mold counts were determined by using the acidified Potato Dextrose Agar, incubated at room temperature for 4 days. The enterococcus counts were determined by using KF Enterococcus Agar with Sodium Azide [inhibitor of micro-organisms other than Enterococcus] incubated at 37 C for 48 hours. The Coliform counts were determined by using Violet Red Bile Agar, incubated at 32 C for 24 hours. The microbiological analysis was conducted to check for the effect of the Patented procedure on inactivating these indicator organisms, which will act as carriers or mechanical vectors of the Omicron virus as well as its naked RNA to infect people to cause Covid-19 disease.

The results of the rapid antigen tests, molecular RNA detecting RT-PCR tests to check for Omicron variant of the SARS-CoV-2 Virus, and the microbiological tests are presented under results and discussion section of this article.

EXPERIMENT-4: The effect of the Patented system on inactivation of the DNA viruses [Bacteriophage], and also their naked DNA, confirmed by using standard plaque assay, and Electron Microscopy.

Sample Preparation: For every 10 ml of the sterile distilled water, Streptococcus thermophilus bacteriophage was added to arrive at approximately10,000 phage particles/ml. Two sets were prepared, one to check for the effect of the test procedure as outlined in the experiment 1 [with inclusion of DNASE and RNASE in sample #3], and second one to check the effect of the test procedure if DNASE and RNASE enzymes excluded from the sample #3. Since the samples were prepared using distilled water, after step-1 and step-2 of the procedure, the incubation period was reduced to 1-5 minutes, as opposed to 30 minutes in Experiment 1.

Bacteriophage counts were determined by running plaque assay before treatment [control], after step-2, and finally after

step-3 [with the inclusion of DNASE and RNASE] of the procedure. Additionally, the phage counts were also determined using the third sample without the inclusion of DNASE and RNASE enzyme. Finally, after finishing the third step of the treatment with and without inclusion of the nuclease enzymes, 1 ml of the samples were inoculated into 100 ml each of sterilized [autoclaved] 12 percent reconstituted Non-Fat Dry Milk inoculated with one milliliter of the thermally injured [not killed] Streptococcus thermophilus bacteria [host specific to DNA bacteriophage used in the experiment] and incubated at 37 C for 12 hours. The procedure to prepare heat injured S. thermophilus was followed as per the instructions presented in the US Patent # 11,643,641 B2. As a positive control the thermally injured S. thermophilus and active S. thermophilus [Host] bacterium inoculated milk and S. thermophilus bacterium inoculated milk plus the untreated bacteriophage were also incubated at 37 C for 12 hours. Wherever applicable, at the end of the incubation, pH of the samples was determined, and also all the control and test samples were analyzed to determine the concentration of the bacteriophage using plaque assay.

In addition, all the above test samples were inoculated into the sterilized 12 % reconstituted Non-Fat dry Milk along with the active S. thermophilus [host] and incubated at 37 C for 12 hours. At the end of incubation both the pH and the bacteriophage titers were determined to assess the efficacy of the patented procedure to inactivate the DNA viruses, including their naked DNA. The results of this experiment are presented in the Results and Discussion section

EXPERIMENT-5: Experimental demonstration of the ability of the naked DNA of the Streptococcus thermophilus bacteriophage to infect bacterial host and successfully produce virus particles,

In this experiment, S. thermophilus bacteria [host] and its specific phage were included. The viral [Bacteriophage] naked DNA was prepared using the detailed procedure outlined in the US patent 11,643,641. The partially injured host bacterium [S. thermophilus] was also prepared by using the procedure outlined in the patent. The heat injured host bacteria [S. thermophilus] was inoculated into two separate bottles containing sterile reconstituted 12% solids of Non-Fat Dry milk. Bottle # 1 was inoculated with viral naked DNA without any further treatment. Bottle #2 was inoculated with naked DNA treated using preparation #3 [with DNASE and RNASE enzymes] as presented in experiment 1. Bottle #3 was inoculated with naked DNA into already inoculated active S. thermophilus host bacterium. All the three bottles were incubated at 37 C for 12 hours. The bacteriophage counts were determined at the end of incubation. The samples were prepared for phage analysis using the test procedure outlined in Patent # 11,643,641. One ml. of the three preparations [at the end of incubation] were inoculated into 100 ml. sterile milk with active host bacterium and incubated for 12 hours at 37 C to check for the propagation of the virus emerged from Naked DNA only [without the outer proteins on head and tail of the bacteriophage]. The results of these experiments are presented under the Results and Discussion section.

Electron Microscopy was also conducted to check for

the liberation of naked DNA from the active S. thermophilus bacteriophage, using both the positive and negative controls. The detailed electron micrographs of the S. thermophilus bacteriophage with intact DNA in its head [a complete virus] and bacteriophage after ejecting the DNA [virus without DNA in its head] as naked DNA are presented in the results and Discussion section.

Results and Discussions

Results of Experiment-1:

The SARS-CoV-2 virus positive saliva samples, when subjected to the test outlined in Experiment-1, tested negative for the RT-PCR test. The samples taken after the second step of the treatment procedure tested positive for the presence of corona viral RNA using RT-PCR test. Turn around, the samples taken after the third step tested negative for the RT-PCR test. It indicates that SARS-CoV-2 viral RNA was still intact after step 2, tentatively proving that the viral RNA was not inactivated. It was inactivated after the third step, since the solution employed in step-3 has RNASE enzyme. Since the RNA was hydrolyzed, the Reverse Transcriptase enzyme could not generate DNA off the viral RNA, to be read by the RT-PCR test. The fact that without the use of RNASE enzyme the saliva samples were still positive for RT-PCR test, indicates that the Coronaviral naked RNA will have capability to induce Covid-19 disease, if it can be introduced into the human cell with the aid of mechanical vectors either through partially damaged cell membrane or through the endocytosis pathway mimicking as a part of nutrients such as amino acids and peptides., due to its smaller size.

It has been well documented and advertised that the SARS-Cov-2 virus loses its Spike proteins and Membrane when it is outside the human body for longer than 3 days. Apparently, it was taken for granted that the viral RNA was also inactivated. Thus, it was concluded by the scientific community including CDC and WHO that the Coronavirus gets ineffective and could not infect after it loses its integral structures such as Spikes and Membranes. In a way this assumption is true, since such a SARS-CoV-2 virus without Spike proteins and membranes will test negative while trying to enumerate using the standard viral enumeration techniques. Previous investigators totally ignored the concept that the corona viral naked RNA can also induce Covid-19 disease, if it is introduced into the human cell through the aid of any mechanical vectors. This experiment proved that RNASE enzyme totally deranged the Coronaviral naked RNA, proven by the fact that the Reverse Transcriptase enzyme could not transcribe the damaged or enzymatically hydrolyzed RNA into DNA, which was to be polymerized to be read in the RT-PCR test.

Results of Experiment-2:

The results of the Field trial conducted in the year 2021 and 2022 proved that the patented system when applied in the construction area, along with other precautions, significantly reduced the Covid-19 infection among the construction workers. According to the earlier report by the management several workers were reinfected by SARS-CoV-2 virus and its variants even though they were vaccinated. Even the individual who took booster shots came down with Covid-19 again.

After instituting the Patented procedure, the rate of Covid-19 infection came down significantly to the tune of roughly 90%. Even the 10 % workers who exhibited the symptoms of Covid-19 such as cough, sneezing and tiredness etc., upon testing using RT-PCR test were found negative. Their symptoms could have been due to long-Covid. Surprisingly none of the construction workers who were vaccinated got reinfected ever since the Patent procedure was instituted to inactivate the SARS-CoV-2 virus and its naked RNA. The results clearly indicate that perhaps Covid-19 coronavirus lineage has intrinsic ability to reinfect people even with aid of naked RNA. Since most of these practical experiments were conducted in late 2021 and early 2022, it can be concluded that the predominant Corona viral variant at that stage of the Pandemic was Delta and even partly some Alpha variants of the SARS-CoV-2 lineage. According to the management, the surface smears were also negative for the SARS-CoV-2 virus checked on the basis of RT-PCR test after instituting the patented procedure. Apparently, the viral RNA could have been digested by the RNASE enzyme used in the Patented procedure. The RT-PCR test only reads negative if the viral RNA genetic sequence is deranged to the point of not being recognized by the Reverse Transcriptase enzyme to convert to DNA. Earlier, prior to instituting the Patented system to inactivate SARS-CoV-2 along with its naked RNA, management reported that most of the surfaces in their work areas tested positive for SARS-CoV-2 virus using RT-PCR test. Ever since they instituted the novel Patented procedure outlined in US Patent # 11,643,641 B2, the environment was free from the SARS-CoV-2 virus including its naked RNA.

One of the reason for the repeated Covid infections, even after the patients were cured and vaccinated, was perhaps due to on-set of Long Covid. Although several medical professionals are perplexed with the etiology of long Covid, from these experimental field trials it can be concluded that perhaps it may be due to the SARS-CoV-2 viral naked RNA prevalent in the environment and thus reinfecting the people or it may be also due to the presence of the naked RNA in the patient for longer period of time, even after Covid- 19 infection is totally cured.

Results of Experiment-3:

The reason for conducting this experiment was to check the effect of the patented system [US patent # 11,643,641 B2] on inactivating both the Nucleocapsid protein and also the naked RNA of the Omicron variants and sub variants of the SARS-CoV-2 lineage. According to the CDC and other agencies and Universities, the only variants and sub variants causing Covid-19 in the year 2023 belongs to Omicron and its descents. In our earlier field trials, the predominant SARS-CoV-2 lineage variants belonged to Delta. In this particular experiment we have confirmed that Omicron variants cause severe symptoms as Delta in patients with comorbid conditions, irrespective of their age groups.

When the Covid-19 positive saliva samples were subjected to the procedure outlined in experiment 1, the rapid antigen test was negative, yet the RT-PCR test was positive after the second step of the test. However, both these tests were negative at the end of the third step of the test. This is an extremely important observation in that the first and second step of the procedure denatured the corona viral nucleocapsid protein to the point that it was not detected by the SARS-CoV-2 antibody present in the Covid-19 Antigen Rapid test materials. Although the iHealth Covid-19 Antigen Rapid Test is designed to check the Antigen present in the Nucleocapsid protein, i can presumably conclude that even the Spike, Membrane, and envelope proteins could have also been denatured or oxidized to the point of significant alteration in viral antigenicity. However, it is of great significance in that the sample obtained after second step was positive for the RT-PCR test indicating step1 and step2 did not alter the genetic structure of the viral RNA. Yet, after the third step of the treatment, both the rapid antigen test as well as RT-PCR tests were negative indicating that the RNASE enzyme digested or hydrolyzed or altered the genetic sequence or the nucleotide arrangement of corona viral RNA to the point that even the Reverse Transcriptase enzyme could not transcribe the viral RNA to DNA in the RT-PCR test.

Furthermore, when both the RNASE as well as DNASE were deleted from solution #3 of the test protocol and then conducted the molecular RNA test, the samples after the third step were positive for the RT-PCR test, indicating that the viral RNA was active and intact. This fact has been ignored by all the previous investigators. Such a naked RNA can infect and cause Covid-19, if it is introduced into human cell either through the disrupted cell membranes with the aid of mechanical vectors such as yeast, molds, bacteria, and even dust particles etc. or through endocytosis along with the nutrients entering into the cell. These results clearly proved that the naked RNA can still be intact, even though the viral proteins and membranes are denatured and disrupted. Thus, one cannot take it for granted that SARS-Cov-2 virus was inactivated and cannot thus

infect humans, due to mere disruption of the nucleocapsid, spike, membrane, and envelope viral proteins.

In this experiment, we have also determined the concentration of the indicator organisms like Yeast and molds, Enterococcus, and Coliforms in the Covid-19 positive patient's saliva, before and after using the test procedure as outlined in experiment 1. The results proved that the test procedures outlined in the US Patent # 11,643,641 B2 completely inhibited the Yeast and molds, Enterococcus bacteria and the coliforms. Any of these mechanical vectors can carry the naked Omicron viral RNA to infect other human beings. The test procedure confirmed that such mechanical vectors can also be inactivated using the novel discovery. This is of the great significance in that some of these micro-organisms may be responsible for the hospital acquired infections [nosocomial infections, which are killing over million people annually and projected to kill close to 10 million people by the year 2050]. This experiment proved that the novel Patented procedure outlined in the US patent # 11,643,641 B2 not only inactivate Coronavirus through denaturation of viral proteins and the RNA [including naked RNA] but also the microbial origin mechanical vectors, which can spread the Covid-19 infection through viral naked RNA. The results of these experiments are presented in Table 4. For the sake of further understanding, a detailed pictorial presentation being presented [figures 3 to 9], in the later part of this section to show the intact Corona viral structure, mechanism of the Covid-19 viral infection, structure of the viral naked RNA, and the hypothetical mechanism by which the mechanical Vectors can propagate the Covid-19 infection by carrying both the intact virus, as well as the naked viral RNA (Table 4).

 Table 4: Effect of the Patented Procedure (US Patent #11,643,641 B2)

 on Inactivation of the Mechanical Vectors and Viral Proteins (Antigen

Male	subject	Female Su	bject
Re	esults	Result	s
Before using Patented Proce- dure (Control)	After Using Patented Procedure (Test)	Before Using patented Procedure (Control)	After Using Patented Proce- dure (Test)
Microbiological Tests	Microbiological Tests	Microbiological Tests	Microbiological Tests
Yeast and Molds ¹ 1000/Ml.	Yeast and molds- < 10/Ml.	Yeast and Molds-300/Ml.	Yeast and Molds- < 10/Ml.
Coliforms- 400/ Ml. ²	Coliforms- < 10/Ml.	Coliforms- 230/Ml.	Coliforms- <10/Ml.
Enterococcus – 300/Ml. ³	Enterococcus- <10/Ml.	Enterococcus- 280/Ml.	Enterococcus- <10/Ml.
Antigen Tests ⁴	Antigen Tests	Antigen Tests	Antigen Tests
+ve	-ve	+ve	-ve
<u>RT-PCR Test</u> ⁵	<u>RT-PCR Test</u>	<u>RT-PCR Test</u>	<u>RT-PCR Test</u>
+ve	-ve	+ve	-ve

Test) and the Naked RNA of the Omicron Variants (RT-PCR Test).

1 - Yeast and Molds- Conducted by using acidified Potato Dextrose agar

2 - Enterococcus Enumeration done by using KF streptococcus Agar [using Sodium

Azide as inhibitor of other bacteria]

3 - Coliform Counts obtained by using Violet Red bile agar.

4 - Antigen Test for SARS-COV-2 was conducted using iHealth Covid-19 Antigen rapid Test.

5 - RT-PCR Test- Reverse Transcriptase- Polymerase Chain Reaction

Results of the Experiment #4

The detailed results of Experiment # 4 are presented in Table 5. The test proved that the DNA Virus was inactivated at the end of step 2 and step 3 using the procedure outlined in Experiment 1. Two variables in the composition of the solution used in step 3 that is with and without the inclusion of DNASE and RNASE enzymes

revealed that the naked DNA gets inactivated with the aid DNASE Enzyme, proven by the fact that the viral titer was < 10/ml. The final product after treating at the 3rd step with the inclusion of DNASE and RNASE, was designated as Sample A. Whereas, the final product after treating at the 3rd step without the inclusion of DNASE and RNASE enzyme was designated as Sample B. Even though Sample A and B both revealed zero survival of the DNA virus using the standard plaque assay, it does not mean that the viral DNA was inactivated. It may merely mean that the viral appendices such as head, tail, tail fiber proteins might have been denatured, thus the standard viral test was negative for the presence of the bacterial DNA virus.

 Table 5: The effect of the novel procedure on inactivating Streptococcus thermophilus DNA phage and its naked DNA.

Variable	Bacteriophage Counts
Initial Phage Count before the test	120 x 10 ⁴ /ml.
Test sample at the end of second step	< 10/ml.
Test sample at the end of third step with the inclusion of DNASE and RNASE- designated Sample A	< 10/ml.
Test sample at the end of third step without DNASE and RNASE designated as Sample B	<10/ml.
Sample A inoculated into specific heat injured bacterial host and incubated at 37 C for 12 hours and designated as Sample C.	< 10/ml. (pH-5.3)
Sample B inoculated into specific heat injured bacterial host and incubated at 37 C for 12 hours and designated as Sample D	20,000/ml. (pH-5.9)
Sample C inoculated into specific active bacterial host (not heat injured) and incubated at 37 C for 12 hours	<10/ml. (pH- 4.8)
Sample D inoculated into specific active bacterial host (not heat injured) and incubated at 37 C for 12 hours	650 x 10 ⁷ /ml. (pH-6.20)

This puzzle was answered by inoculating sample A and sample B into reconstituted 12 % solids Non-Fat dry milk which was inoculated with the heat injured viral host bacteria Streptococcus thermophilus and incubated for 12 hours at 37 C. The sample A inoculated milk with the heat injured host was designated as sample C and the sample B inoculated milk with heat injured host was designated sample D. The reason for using the heat injured, but not killed, host is to help to integrate the viral naked DNA [if present in sample A and B] to infect the bacterium to produce the healthy viral progeny, without the aid of appendices. The results presented in Table 5 revealed that Sample A [where DNASE and RNASE enzyme were added at the 3rd step, which is then inoculated with the thermally injured host is designated as sample C] tested negative for the presence of the bacteriophage [Final pH after incubation -5.30], indicating the viral naked DNA was totally inactivated using the invention outlined in the US Patent # 11,643,641 B2. Whereas sample B [without the inclusion of DNASE and RNASE], tested positive for the bacteriophage with a titer of 20,000 phage particles/ ml, [Final pH after incubation-5.9]. It indicates that an intact naked viral DNA can infect the host, without the aid of intact head, tail, and other appendices. I can unequivocally mention that it is the first time in the world that such a mechanism was demonstrated regarding the capability of the naked DNA to infect the host under

certain circumstances. We can presume and hypothesize that such mechanism can also operate in the DNA viruses which infect humans, necessitating the dire requirement to not only to inactivate virus [demonstrated by the available laboratory test procedures to check for viability] but also its naked DNA to protect humanity from viral infections.

To further prove that the virus produced by the naked DNA are still capable of infecting the parent active host, the samples Cand D were inoculated along with the active host as outlined under materials and methods section were incubated at 37 C for 12 hours. The results revealed that sample C did not inhibit host bacterium, proven by the fact that pH of the milk drop to 4.8, and the titer by plaque assay was <10/ml. Whereas the sample D totally inhibited the host [pH 6.20] with viral titer of 650 x 107 /ml [6.5 billion/ ml.] indicating viral naked DNA can produce virions with equal capability as its original virulent parent. It is also proven beyond doubt that the Patented novel procedure outlined in the US patent # 11,643,641 B2 is a breakthrough invention demonstrating the fact that the naked viral DNA must be inactivated to totally curb viral multiplication. This experiment has great significance in explaining the reason why some of the viral Pandemics are hard to contain despite the advances in the modern sanitation procedures and sanitation compounds.

Results of Experiment -5

Since Experiment 4 did not include the isolated viral naked DNA, this experiment was specifically designed to demonstrate the capability of the isolated viral naked DNA to infect the host to propagate the progeny, under certain conditions. The results of this experiment are presented in Table 6. The results revealed that the sample one where the naked DNA, which has not been treated with DNASE in the procedure, when inoculated into the sterile milk with heat injured Streptococcus thermophilus bacterium [Host] was able to produce virions at the end of incubation. The viral titer was 120x 10²/ml [12,000/ml.]. This was designated as Sample A. The second sample where the viral naked DNA was treated using DNASE enzyme, as outlined in the third step of Experiment 1, no viral particles were detected, indicating the test procedure totally deactivated the naked viral DNA. This was designed as sample B. The third sample where the viral naked DNA was inoculated into milk impregnated with the active Streptococcus thermophilus [Host] yielded only 20 phage particles per ml. indicating the infection rate by the naked DNA was significantly low when the host bacteria was active and structurally intact, as opposed to the heat injured host bacterium. This was designated as sample C.

When all the three samples were chloroform treated to inactivate the host bacterium but not the virus and then inoculated separately into three sterilized reconstituted 12 % solids of Non-Fat Dry Milk along with the active Streptococcus thermophilus host and then incubated at 37 c for 12 hours, the sample A preparation completely inactivated the host bacterium with a titer of 400 x 10⁷ /ml active virus particles. Whereas Sample B did not inhibit the host bacterium and no virus particles were detected, indicating the novel patented procedure outlined in US patent # 11,643,641 totally inactivated the viral naked DNA. Whereas Sample C exhibited the partial inhibition of the host bacterium with a final viral count of 130 x 10² /ml. The logical explanation for this phenomenon is due to the low concentration of the virus in the inoculum, to start with. Perhaps upon further transfer viral concentration will be significantly greater.

The results of this experiment clearly proved that the naked viral DNA could infect the host, even though it is devoid of the required viral structures, under certain circumstances. Also, it is proven beyond doubt that the discovery outlined in US patent # 11,643,641 B2 is very effective in hydrolyzing or altering the integral structure of naked DNA. This has great significance in that even the human viral naked DNA can also infect humans if it can be introduced through the aid of mechanical vectors or through endocytosis along with nutrients such as amino acids and peptides etc (Table 6).

in accordance with US Patent # 11,643,641 B2.

 Sample #
 Description Of The Variable
 Viral (Phage) Count at the End Of Incubation

 1
 Naked viral DNA (not treated with DNASE) inoculated into sterile milk along with the heat injured host Streptococcus thermophilus and incubated- designated as Sample A
 120 x 10 ²/ml.

Table 6: The Effect of Viral Naked DNA to infect and propagate its progeny, and the Effect of the DNASE Enzyme to Totally Inactivate the Naked DNA.

	Sample A	
2	Naked specific viral DNA (treated with DNASE) inoculated into sterile milk along with the heat injured host Streptococcus thermophilus and incubated- designated as Sample B	< 10/ ml.
3	Naked specific viral DNA inoculated into sterile milk with active host-Streptococ- cus Thermophilus and incubated-designated as Sample C	20/ml.
4	Sample A (after inactivating residual bacteria but not virus) inoculated into sterile milk with active Streptococcus thermophilus and incubated	400 x 10 ⁷ /ml
5	Sample B – similar protocol as Sample #4	< 10/ml
6	Sample C- similar protocol as Sample # 4	$130 \ge 10^2 / ml$

The results of all the experiments clearly proved that the procedures outlined in the novel US Patent # 11,643,641 B2 were highly reproducible. It was proven that the naked RNA of SARS-Cov-2 viral lineage can survive in the environment or for that matter can survive even after instituting the ordinary cleaning procedures. It was proven through experimentation [experiments 1, 2, and 3] that the ordinary clean up using the surfactants and sanitizers will affect only the proteins of the Coronavirus, including nucleoproteins, Spike, Membrane, and envelope proteins but not the inner RNA, thus exposing the viral RNA as naked RNA. Since Surfactants and Sanitizers can denature the viral proteins, the antigen test was negative. Thus, the altered antigen was not detected by the specific antibody produced against the SARS-CoV-2 nucleocapsid protein. It was highly significant in that although the viral proteins were

denatured and thus were negative for the Antigen detection test, the molecular RT-PCR test was positive indicating that SARS-Cov-2 RNA was not affected by the Surfactants and Sanitizers. It was further proven by the fact that in the last step [3rd Step] of the treatment protocol, the viral naked RNA was hydrolyzed and thus was rendered genetically inactive to produce any further viral progeny [proven by the RT-PCR test], even if it is introduced by the mechanical vector[s] into human cell.

Although it is very hard to conduct such experiments using these pathogenic viruses in humans, especially considering the Pandemic, it has been proven for the first time that such a mechanism does exist in the non-pathogenic DNA viruses infecting bacteria. In experiments 4 and 5, we have proved beyond doubt that naked DNA like naked RNA, can infect the bacteria, under certain conditions, to propagate their viral progeny. In my opinion, such an observation had never been made before by any investigator in the world. It has been proven beyond doubt that merely inactivating viruses through inactivation of their surface infective proteins does not guarantee that the virus lost its capability to infect and elicit the infection. In order to totally inactivate the virus, one must inactivate not only the surface proteins but also the viral genetic materials RNA and/ or DNA. This research publication based on the discovery outlined in the US patent # 11,643,641 B2 can be considered a breakthrough in the field of Virology in order to explain the possible reason why we could not contain the Covid-19 Pandemic, and for that matter even the yearly repeated common flu due to influenza viral lineage (Figure 1 and 2).

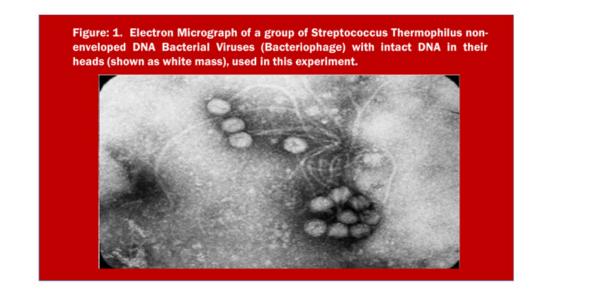


Figure 1: Electron Micrograph of a group of streptococcus thermophilus non-enveloped DNA Bacterial Viruses (Bacteriophage) with intact DNA in their heads (shown as white mass), used in this experiment.

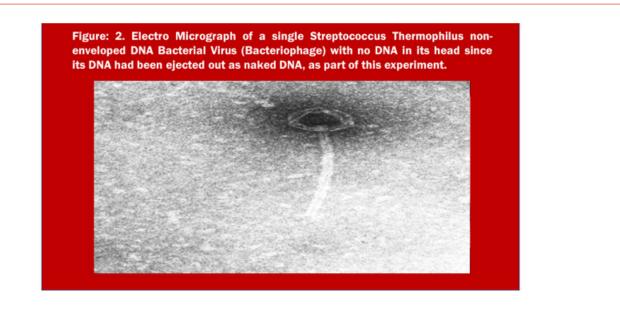


Figure 2: Electro micrograph of a single streptococcus thermophilus non-enveloped DNA Bacterial Viruses (Bacteriophage) with no DNA in its head since its DNA had been ejected out as naked DNA, as part of this experiment.

Using this approach, we can control future unexpected pandemics due to viruses as well as pathogenic bacteria. This novel discovery further proved that all the Coronaviral SARS-CoV-2 variants such as Alpha, Beta, Gamma, Delta, and Omicron etc. can be totally inactivated [including their naked RNA] to contain the

Pandemic.

This discovery and research has greatest significance in that it is one of the greatest tools to inactivate even the hospital acquired infections also, which are killing over million people per year, and which are projected to kill over 10 million people per year by the year 2050. Apparently, these Nosocomial infectious agents not only cause incurable bacterial diseases such as C. diff., and MRSA etc., but also act as carriers to the intact viruses as well as their viable naked Genetic materials such as naked RNA and/or naked DNA, to cause severe viral infections such as Covid-19.

To further illustrate how these naked RNA can infect the human cells through the aid of mechanical vectors, I am presenting a series of pictorial presentations in this research article. Tables 7 and 8 shows the relative sizes of the Corona virus [SARS-CoV-2] and its naked RNA in relation to the size of the mechanical vectors such as Molds, yeasts, Mold spores and the bacteria. One can appreciate

how small the size of the virus and their naked RNA in comparison to the vectors. The popular figure 3 [courtesy of CDC] shows the integral structures of the SARS-CoV-2 virus especially pointing to the locations of the Spike, Membrane, envelope, and Nucleoproteins and also the core viral genetic material-RNA. It is the Spike protein that helps the Coronavirus to make a contact with human cell through interaction with ACE-2 receptors. Most of the vaccines are produced to generate antibodies, which can attach to Spike proteins to eliminate the infection by the Coronavirus. Unfortunately, due to extensive mutations observed in the SARS-CoV-2 genetic material RNA, the variants have different antigenicity in the Spike protein thus overriding the effect of vaccine induced or produced antibodies [29,30,31].

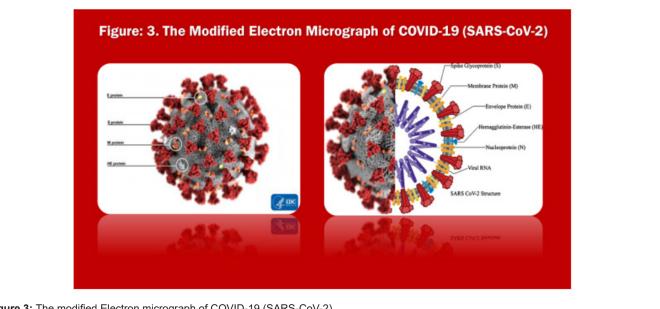


Figure 3: The modified Electron micrograph of COVID-19 (SARS-CoV-2).

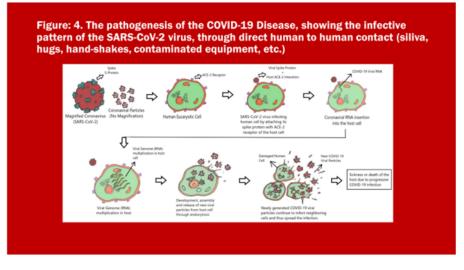


Figure 4: The Pathogenesis of the CoVID-19 Disease, showing the infective pattern of the SARS-CoV-2 virus, through direct human to human contact (siliva, hugs, hand-shakes, contaminated equipment, etc.).

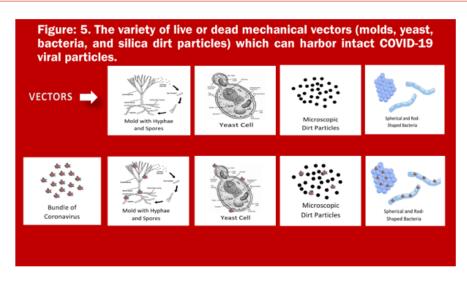


Figure 5: The variety of live or dead mechanical vectors (molds, yeast, bacteria, and silica dirt particles) which can harbor intact COVID-19 viral particles.

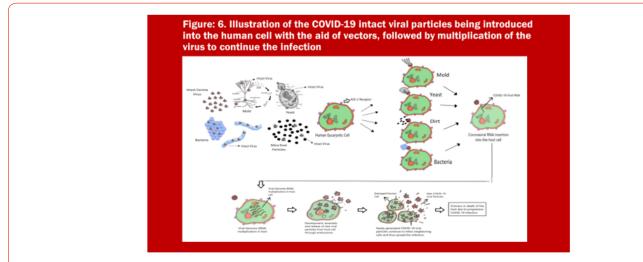


Figure 6: Illustration of the COVID-19 intact viral particles being introduced into the human cell with the aid of vectors, followed by multiplication of the virus to continue the infection.

Figure: 7. The fate of COVID-19, SARS-CoV-2 virus while it is outside the human body for an extended period of time (over one week), showing the release of viral naked RNA through disintegration of the anatomical structure.

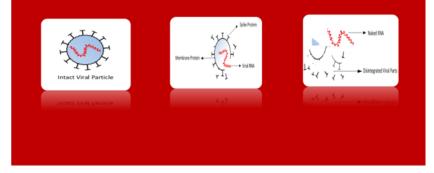
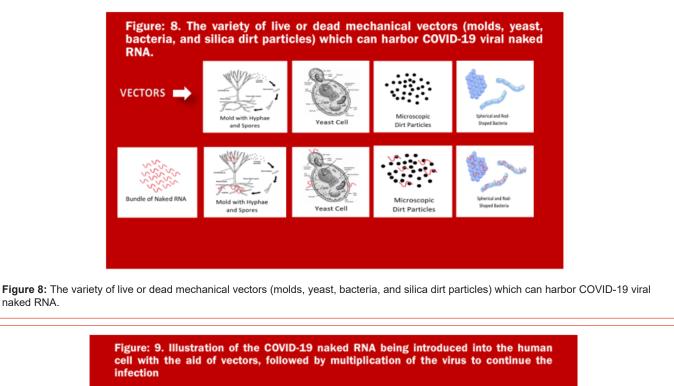


Figure 7: The fate COVID-19, SARS-CoV-2 virus while it is outside the human body for an extended period of time (over one week), showing the release of viral naked RNA through disintegration of the anatomical structure.



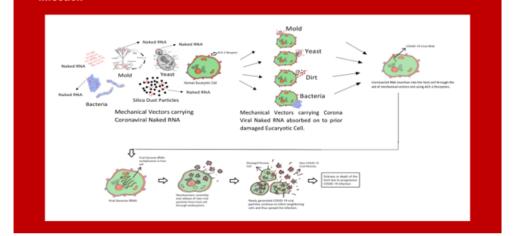


Figure 9: Illustration of the COVID-19 naked RNA being introduced into human cell with the aid of vectors, followed by multiplication of the virus to continue the infection.

Figure 4 shows the pictorial presentation of how SARS-CoV-2 Corona virus, and its variants infect the human cell to propagate [through endocytosis- budding process] their viral progeny to induce Covid-19 disease. Figure 5 shows various mechanical vectors and their morphology to act as carriers of the SARS-CoV-2 virus. Figure 6 depicts the mode of transfer of the SARS-CoV-2 by the mechanical vectors to infect and propagate in the eukaryotic cells to induce Covid-19 infection. Figure 6 demonstrates the fate of Corona Virus, while it is outside the human body to liberate the viral naked RNA. Figures 8 and 9 clearly demonstrate how the viral naked RNA can be transferred with aid of mechanical vectors, to assist the viral multiplication to induce Covid-19 infection. Now the reader can appreciate the importance of inactivating the SARS-CoV-2 viral naked RNA to contain the Pandemic. Perhaps the SARS-CoV-2 naked RNA could have been the culprit for the spread of Covid-19 pandemic, which could not be contained. In all probability the long Covid, which has affected over 23 million people in Us and approximately over 100 million people around the world ca also be due to the SARS-CoV-2 viral naked RNA (Table 7,8) (Figure 3-9). Table 7: Measurements of COVID-19 Virus and its naked RNA in comparision to their vectors, mold, yeast, bacteria, and mold spores, etc.

Biological Entity Type	Size of the Organism in Microns and Nanometers
Mold Hyphae	2000 to 5000 Microns
Mold Spore	2 to 5 Microns
Spherical Bacterium	1 to 5 Microns
Coronavirus	0.02 to 0.1 Microns or 20 to 100 nanometers
Naked RNA of Coronavirus	2 Nanometers

Unit measurements:

Micron (.001 MM) or 1.00 Micrometre is equal to 1 millionth of a meter, represented as μM

· nanometre or millimicron is equal to 1 billionth of meter, represented as mµ

Table 8: Relative size of the coronavirus and coronaviral naked RNA in comparison to vectors mold hyphae, bacterial cell, yeast cell, and mold spore.

Coronavirus is 10,000 times smaller than single mold hyphae
Coronaviral naked RNA is 1,000,000 times smaller thanmold hyphae
Coronaviral naked RNA is 10,000 times smaller single bacterial cell
Coronaviral naked RNA is 50,000 times smaller than single yeast cell
Coronaviral naked RNA is 10,000 times smaller than one single mold spore

For the sake of the reader, I am submitting some of the published facts about naked viral genetic materials [synthesized in the laboratory] which can infect the host, without having the outer structures of the intact virus.

The following are the published facts [done using synthesized DNA], along with the author's [MS Reddy's] comments, regarding the infectious abilities of Naked genetic materials I.e., Naked RNA/DNA to infect and induce viral infection without the aid of structurally intact parent virus particle with enclosed RNA/ DNA.

A published paper titled, unregulated hazards and free nucleic acids [naked RNA/DNA], is a conference paper from two decades ago, first circulated at Biosafety meeting held in Montreal, Canada [Jan 24-28, 2000]. This paper is available online in its entirety at http://twn.My/title/naked.htm. The following facts [1-9] are published regarding the hazards associated with free nucleic acid [naked RNA/DNA], followed by the author's comments. For the sake of the reader's convenience, I have pointed out the lines and paragraphs of the original published paper on this controversial issue supporting the hypothesis and proven discovery outlined in the U.S. Patent #11,643,641B2, along with the relevant comments by the author Dr. M.S. Reddy as it applies to the current subject of the coronaviral naked RNA and its infective ability to illicit COVID-19 disease.

1. The lack of regulation of naked/free nucleic acids is based largely on the assumption, now proven to be erroneous, that the naked/free [naked RNA/DNA] would be rapidly broken down in the environment and in the digestive system of the animals. Another false assumption is that as DNA is present in all organisms, it is not a hazardous chemical, and hence there is no need to regulate it as such [Page 3, last paragraph of the above specified online publication].

AUTHOR'S COMMENT: Naked DNA/RNA will not be broken

down easily, even though they are not inside the viral particle. Thus, they can be infective, even without the aid of the intact virus. The recent 2022 Nobel prize is awarded to a scientist who has isolated DNA from 40,000 years old Neanderthal bones, proving that naked nucleic acids will not be broken down easily as people previously thought.

2. Naked or free DNA are now known to persist in all natural environments, and also in the mouth and digestive tract of mammals, where it may be taken up and incorporated by the cells of the mammalian host [Page 4, paragraph1- below box-2].

AUTHOR'S COMMENT: Naked genetic materials such as viral naked DNA or naked RNA can enter into human cells to multiply and make more viral particles, although the naked genetic material is not intact in the parent virus. Thus, SARS-Cov-2 viral naked RNA can elicit infection if it is introduced into the human cell.

3. It has long been assumed that naked DNA cannot be taken up through intact skin, surface wounds, or the intestinal tract or it would be rapidly destroyed if taken up. The ability of naked DNA to penetrate intact skin has been known at least since 1990. Cancer researchers found that within weeks of applying the cloned DNA [naked DNA] of a human Oncogene to the skin on the back of the Mouse, tumors developed in endothelial cells lining the blood vessels and lymph nodes [Page 4, paragraph 3-below box 2].

AUTHOR'S COMMENT: Naked viral DNA or naked viral RNA [of both Enveloped and Non-enveloped virus] can enter through wounds or broken skin into the human cells and can multiply to generate viruses even though it is free from the intact viral particle.

4. Viral DNA [viral naked DNA which is liberated from the intact viral particle] fed to Mice is found to reach White Blood Cells, Spleen, and Liver Cells via the intestinal wall, to get incorporated into the Mouse Cell Genome. When fed to pregnant mice, the

viral DNA [naked DNA] ends up in the cells of the Fetuses and the newborn animals., suggesting it has gone through the placenta as well [Page 4, paragraph 4- below box 2].

AUTHOR'S COMMENT: The viral naked genetic material can pass through the intestinal tract, to infect the host. Thus, naked viral genetic material of SARS-CoV-2 virus and their mutants cannot be ignored, although the viral particle is not intact or destroyed by the sanitizing agents.

5. One of the key findings is that the naked viral DNA is more infectious and has a wider host range than the intact virus. Human T-cell Leukemia viral DNA formed complete viruses when injected into the blood stream of the rabbits. Similarly, naked DNA from the human polyomavirus BK [BKV] gave a full-blown infection when injected into rabbits, despite the fact that the intact BKV virus is not infectious [Page 5, paragraph 3, lines 1 to 4].

AUTHOR'S COMMENT: The naked viral RNA or viral DNA can cause full-blown infection without the aid of intact infectious virus. Apparently, such mechanism exists in the enveloped viruses, where envelope can be disrupted easily by the disinfectant or sanitizing agents, yet the naked DNA or naked RNA can still infect the host with the aid of mechanical vectors such as live or dead bacteria, yeast, and molds etc. According to the literature such naked genetic material is more infectious and has a wider host range than the intact virus. Perhaps such mechanism may be operative during COVID-19 Pandemic due to SARS CoV-2 virus and its mutants Delta and Omicron. This fact cannot be ignored because we are not able to contain the pandemic and it is getting worse due to viral mutations involving more rate of infection [70% or more] and wider host range infecting children etc., which the original SARS-CoV-2 virus did not do.

6. Foreign DNA can be delivered into mammalian cells by bacteria that can enter into the cells [Page 7, paragraph 3, sub section 5].

AUTHOR'S COMMENT: Bacteria, which can be either live or dead can act as mechanical vectors to carry the naked viral genetic material [naked RNA/DNA] into the human system to induce the full-blown viral infection. Thus, the patented invention outlined in the U.S. Patent #11,643,641B2 is novel in that the invention is aimed to inactivate and digest the cell walls of the mechanical carriers so that they cannot harbor to act as mechanical carriers to carry the naked viral genetic materials to infect the host.

7. Genetically "crippled" strains of bacteria, supposed to be biologically contained, are nevertheless found to survive in the environment and to swap genes with other bacteria. [Page 8, box 6, subsection 5].

AUTHOR'S COMMENT: To eliminate the crippled bacteria, which can survive and act as mechanical vectors, the current invention includes total kill of the bacteria, yeast and molds followed by the digestion of their cell walls etc. to prevent them acting as mechanical vectors to harbor the viral naked RNA or DNA.

8. DNA [naked DNA] from viruses is more infectious than the intact virus itself [Page 9 subsection?]

AUTHOR'S COMMENT: Self-explanatory and this is specifically true with the enveloped viruses, when envelope can be stripped exposing either naked DNA or naked RNA, which can be more infectious than the intact virus itself. This could be the reason for the widespread COVID-19 pandemic with multiple variants of SARS-CoV-2 virus.

9. Unlike chemical pollutants, which dilute out and degrade over time, nucleic acids can be taken up by cells to multiply, mutate and recombine indefinitely. It is irresponsible to continue to exclude naked/free nucleic acids from the scope of the Biosafety products [Page 10, conclusion].

AUTHOR'S COMMENT: The viral naked RNA/DNA has to be completely destroyed using Enzymes to deactivate the genome and to eliminate the spread of viral infections. Overall, it is proven beyond doubt that the viral naked RNA/DNA [naked nucleic acids], which are the main genetic determinants must be completely deactivated to eliminate further spread of viral infections. It is clear that rendering virus ineffective to infect the host cells does not guarantee that the covalent bond DNA and RNA is inactivated. The virucidal effect is only 100 % efficient, provided both the external viral structures, protective membranes, and the integral molecular structure of RNA [perhaps naked RNA] are totally destroyed.

The research-based answers given in the form of written comments by the author clarifies the myths associated with the naked RNA and naked DNA and their abilities to infect the host to cause infection. In a nutshell, the essence of the current research and the patented novel procedure to inactivate both the enveloped and the non-enveloped viruses along with their genetic material [naked RNA/DNA] is as follows: An effective and progressive treatment to reach and inactivate the naked RNA, a detergent effective against viral envelop with a lipid moiety is formulated [the composition of which is presented with details in the current research article] and misted throughout a bounded area and granted a period of repose. This step is followed by an application of an inactivator of the viral spike protein [the composition of which is outlined in the U.S. Patent #11,643,641B2]. In the next step, the threat of naked viral RNA transmission by mechanical vectors has been accomplished by the application of an inhibitor [natamycin, sorbic acid, and sodium propionate] of mold, yeast, and bacteria, followed by the use of enzymes [Alpha Amylase, and Cellulase] to degrade the cell walls of the bacteria, yeast , and hyphae of the molds, so that they no longer can harbor and transmit coronavirus or it's naked RNA. Finally, the exposed naked RNA/DNA is inactivated by the stabilized nuclease enzymes i.e. RNASE and DNASE. The finer details including compositions and percentages of the ingredients are presented earlier in this communication. Further details can be obtained from the U.S. Patent #11,643,641B2.

In my opinion, one of the reasons why the SARS-CoV-2 virus and its variants are not contained during Pandemic, is because of the spread of the viable naked RNA through the mechanical vectors to induce the infection in the host. This invention is novel in that the multiple step procedure inactivates not only the viral structures [envelope, spike proteins etc.] but also its inner isolated or naked RNA/DNA. All of the prior investigations were silent about inactivating the viral genome of even the enveloped viruses, which is naked RNA/DNA. Their definition of inactivation of the enveloped virus is dissolving the viral envelope to make the virus incapacitated to infect the host cell. However, they were silent about inactivating the naked RNA/DNA infecting and multiplying in the host cell if it is introduced into the host cell through mechanical vectors or through the nutrient entry channels of endocytosis. Unless the genome is destroyed, the virus cannot be considered dead. It is only incapacitated, proven by not being able to be enumerated using the conventional laboratory procedures.

This invention is 100% effective not only on enveloped virus, but also on the non-enveloped virus and mechanical carriers such as gram-positive and gram-negative bacteria, and yeast and molds, proven through repeated experiments, which are outlined in this publication.

However, none of the prior inventions or disinfectant compounds were developed to inactivate the naked viral RNA/DNA to cure the spread of the viruses, including the seasonal influenza virus., which are also enveloped RNA viruses. Apparently, to curb these viral infections it is necessary to inactivate their naked RNA/DNA. Merely incapacitating them using disinfectants, without destroying their genetic material is not an effective solution. The discovery outlined in the U.S. Patent #11,643,641B2, and in this current research article has resolved this eternal long lasting problem. Besides, none of the prior inventions attempted to reduce the size of the mechanical vectors [including live/dead bacteria, yeast, and molds] which can harbor and disseminate the viral naked RNA/DNA to infect the host, besides causing the deadly hospital acquired [nosocomial] infections [24,25,26,32].

Conclusion

The novel discovery published in the US patent # 11,643,641 B2 to inactivate the SARS-CoV-2 corona viral naked RNA has been proven effective on all the variants belonging to novel SARS-CoV-2 lineage causing Covid-19. Due to the high mutation rate of SARS-CoV-2 Corona virus, several variants with different antigenicity developed in 2020 [Alpha, Beta etc.], 2021 [Gamma, Delta etc.], 2022 [predominantly Delta], and 2023 [Omicron including EG.5]. Unlike vaccines, irrespective of the mutations resulting in various variants, the patented discovery [US Patent # 11,643,641 B2] inactivated both the surface viral proteins [Spike, Membrane, Envelope, and Nucleoproteins] and also the inner genetic material [viral naked RNA]. The investigation also proved that the mere inactivation of the surface viral proteins is not an assurance of the total viral deactivation, as assumed by prior investigators. The viral naked RNA should be inactivated totally to ensure that the virus is completely destroyed and ineffective to cause viral infection. The methodology outlined in the US Patent # 11,643,641 B2 is also proven to be significantly effective against the mechanical carriers of viruses as well as their naked RNA/DNA, to induce viral infections, including Covid-19. The mechanism of infection by viral naked genetic material DNA, has been demonstrated for the first time, using the Streptococcus thermophilus bacteriophage and its specific host. Various hypothetical mechanisms regarding several

modes of Coronaviral infections due to intact virus and their naked RNA by mechanical carriers has been presented in pictorial form for easy understanding. It is concluded using the experimental Results and evidence that the inactivation of the viral genetic material in total, along with other integral viral structures, in the environment, is the only safest way to control the Pandemic as well as the associated Long-Covid, which is currently affecting over 23 million people in US alone and perhaps over 100 million people around the world. This novel discovery can be applied to curb the seasonal Flu [Influenza] viral epidemic, and the Respiratory Syncytial Virus [RSV], and the hospital acquired infections [nosocomial infections] due to yeast, mold, bacteria and other pathogenic viruses, and any future pandemics due to either enveloped and/or non-enveloped, RNA and/or DNA life threatening viruses, and also perhaps even by the uncontrollable bacterial origin Pandemics.

Disclosure

The author is a scientist heavily involved in probiotic research and holds over 150 US and International patents and published over 160 research articles. His company [IMAC, Inc.] manufactures food-grade and beneficial microbial cultures and other essential high-tech enzyme fortified functional products that go into manufacturing cheese and other dairy products in the United States, Canada, Europe, Asia, and South America.

Acknowledgements

I am extremely thankful and grateful to Mr. V. R. Mantha, director of Quality Control and Research and Development of IMAC [International Media and Cultures], a division of the American dairy and Food consulting laboratories Inc., of Denver, Colorado, USA, for coding and compiling the data, and for helping to prepare this elaborate research article. Deep gratitude to Mr. Rasheed Hussain and Mr. Sridhar Reddy for their contribution to graphs and tables and the original impressive artwork that are presented in this extensive research article. Thanks, are also extended to all the individuals and organizations who participated in the clinical trials including but not limited to patients, technicians, clinical testing laboratories in both USA and India for their cooperation and support while conducting these extensive research trials and experiments.

Conflict of Interest

No conflict of interest.

References

- 1. Reddy MS (2021) Selected Multiphase Treatment for Coronavirus Respiratory Infections. US Patent #11,077,052 B1: 1-31.
- Reddy MS (2021) Mechanism of Thrombosis During COVID-19 Infection Due to SARS-CoV-2 Virus and its Variants, and a Clinically Proven Strategy to Combat with Probiotics and their Immunomodulins. Medical Research Archives, [S.I.] 10(9): 1-21.
- 3. Katella K (2023) What to Know About EG.5 (Eris) the Latest Coronavirus Strain.
- Reddy MS (2023) Prevention of Viral Transmission by Naked Genetic Material US Patent #11,077,052 B1: 1-15.
- 5. Wang P, Nair MS, Liu L (2021) Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7. BioXRiv.

- 6. Wang P, Wang M, Yu J, et al. (2021) Increased Resistance of SARS-CoV-2 Variant P.1 to Antibody Neutralization. BioXRiv.
- 7. Xie X, Liu Y, Liu J, et al. (2021) SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. The Lancet 2(7): E283-E284.
- 8. Garcia-Beltran W, Lam EC, St. Denis K, et al. (2021) Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. Cell 184(9): 2372-2383.
- 9. Reddy MS (2021) Mechanism of Cytokine Storm in COVID-19: How can probiotics combat it? JAPPI 1(3): 27-35.
- Reddy MS (2021) Probiotics: genesis, current definition, and proven therapeutic properties. JAPPI 1 (2): 18-26.
- 11. Reddy MS (2020) Scientific and medical research on Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy and its influence on assisting to cure or prevent the nosocomial infections, synergistically enhancing the conventional cancer therapies, and it's potential to prevent or cure COVID-19 novel coronavirus infection by balancing the intestinal microbiota and microbiome through modulation of the human immune system. Int J. Pharma Sci Nanotech 13: 4876-4906.
- 12. Reddy MS (2019) Genesis, evaluation, and progression of a breakthrough discovery to efficiently cure cancer through use of Dr. M.S. Reddy's Multiple Mixed Strain Probiotics as adjuvants along with the traditional cancer therapies, through restoration of healthy and balanced intestinal microbiota and their microbiome. LOJ Phar Cli Res 1(5): 107-109.
- Reddy MS (2020) Potential for transmission of SARS-CoV-2 infection through its naked RNA: Relevance to health providers. Sushruta Med News AAPI Publication: 3-5.
- Allen H, Vusirikala A, Flannagan J, et al. (2021) Increased household transmission of COVID-19 cases associated with SARS-CoV-2 Variant of Concern B.1.617.2: a national case-control study. Public Health England.
- 15. Wong AC, Devason AS, Umana, IC, et al. (2023) Serotonin Reduction in Post-Active Sequelae of Viral Infection. Cell 186(22): 4851-4867.
- Natarajan A, Zlitni S, Brooks EF, et al. (2020) Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection. Med (NY) 3 371-387.e9.
- 17. Swank Z, Senussi Y, Manickas-Hill Z, Yu XG, et al. (2022) Persistent circulating SARS-CoV-2 spike is associated with post-acute COVID-19 sequelae. Clin. Infect. Dis 76(3): e487-e-490.
- Zollner A, Koch R, Jukic A, Pfister A, et al. (2022) Post acute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases. Gastroenterology 163: 495-506.e8.
- Peluso MJ, Ryder D, Flavell R, et al. (2023) Multimodal Molecular Imaging Reveals Tissue-Based T Cell Activation and Viral RNA Persistence for Up to 2 Years Following COVID 19. Med Rxiv.
- 20. Goh D, Lim JCT, Fernaíndez SB, et al. (2022) Case report: Persistence of residual antigen and RNA of the SARS-CoV-2 virus in tissues of two patients with long COVID.Front. Immunol 13: 939989.

- 21. Singer D, Camargo SMR, Ramadan T, et al. (2012) Defective intestinal amino acid absorption in Ace2 null mice.Am. J. Physiol. Gastrointest. Liver Physiol 303: G686-G695.
- 22. Hashimoto T, Perlot T, Rehman A (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 487: 477-481.
- 23. Zhu X, Levasseur PR, Michaelis KA (2016) Distinct brain pathway links viral RNA exposure to sickness behavior Sci Rep: 629885.
- 24. Reddy MS, Reddy DRK (2016) Isolation and determination of the major principle of causative agent behind the 2016 published breakthrough discovery of Dr. M.S. Reddy's "Multiple Mixed Strain Probiotic Therapy" in successfully treating the lethal hospital acquired infections due to Clostridium difficile (C. diff) and Methicillin Resistant Staphylococcus Aureus (MRSA). Int J Pharma Sci Nano Tech 6: 3556-3566.
- 25. Reddy MS, Reddy DRK (2017) An insight into the 2016 Best Medical Award-Winning Breakthrough Microbial and Nanotechnology based discovery of Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy, to successfully treat the Nosocomial infections. Nano Technol Nano Sci 1(1): 2-5.
- 26. Reddy MS (2018) Immunomodulatory effect of "Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy" to cure or prevent hospital acquired nosocomial infections due to Clostridium difficile (C. diff), other pathogenic bacteria, and autoimmune diseases. Int J Pharma Sci Nano Tech 11:3937-3949.
- 27. Reddy MS (2018) Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Adjuvant Cancer Therapy, to complement immune check point therapy and other traditional cancer therapies, with least autoimmune side effects through eco- balance of human microbiome.
- 28. Moura IB, Buckley AM, Wilcox MH (2021) Can SARS-CoV-2 be transmitted via faeces? Current Opin Gastroenterol 38 (1): 26-29.
- 29. Zhou B, Thi Nhu Thao T, Hoffmann D (2021) SARS-CoV-2 spike D614G change enhances replication and transmission. Nature 592: 122–127.
- Volz E, Hill V, McCrone J, et al. (2021) Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on Transmissibility and Pathogenicity. Cell 184: 64-75.
- Korber B, Fischer WM, Gnanakaran S, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell 2020; 182: 812-827
- 32. Reddy MS, Reddy DRK (2016) Development of Multiple Mixed Strain Probiotics for "Probiotic therapy" under clinical conditions, to prevent or cure the deadly hospital acquired infections due to Clostridium difficile (C diff) and Methicillin Resistant Staphylococcus aureus (MRSA). Int J. Pharma Sci Nanotech 9: 3256-3281.