

Modernized Variolation

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ABSTRACT AND PREAMBLE

Rapid growth in the power of frontier AI models and their potential to enable design of sophisticated bioweapons demands the urgent research and development of rapidly deployable pathogen countermeasures. One such countermeasure class is modernized variolation (MV), in which a pathogen-containing sample is obtained from an infected person and treated using modern technologies to render it largely harmless yet still capable of eliciting both innate and adaptive immune responses similar to those resulting from modern vaccination or even infection itself. This document outlines the basic requirements for such a technology for small-scale use, e.g., among people living under the same roof, as well as the community and organizational elements required to establish the infrastructure needed for a reliable MV-based defense against most pathogens.

This is an early white paper draft; it is intended to be a foundation for Radvac community discussion, brainstorming, and testing of various elements of a successful MV strategy. We on the Radvac core team greatly appreciate the thoughts of the greater Radvac community—and your valuable contributions over the years. Many people in the community have countless times before extended and expanded our initial thinking, and have engaged in highly informed and thoughtful self-experimentation to improve upon our efforts. We hope the community will make similar contributions to this effort, leading to detailed, effective, safe—and widely used—MV protocols.

At the end of this document is an appendix consisting of an overview of three case studies (the full narrative of the first case study is also published [here](#) on Substack) describing development of practical MV protocols and initial testing of the MV approach.

This document also contains detailed pilot protocols for sample collection, pathogen inactivation by hydrogen peroxide, and self-administration by four different methods: intranasal, transdermal, oral cavity via dental floss, and oral administration of an enteric capsule. Results from the use of these protocols seem promising, but much needs to be done to optimize them and show that the overall MV approach works as intended.

The need for speed

There is growing concern among experts that rapid growth in the power of frontier AI models is substantially increasing the risk of bioweapon design and deployment.^{1,2,3} The SARS-CoV-2 pandemic caused a worldwide shutdown despite a relatively low mortality rate of about 2% or less. There are many known pathogens with much higher mortality rates, including SARS (10%) and MERS (35%) coronaviruses. A May 2026 outbreak of hantavirus on a cruise ship was caused by the Andes strain, a variant that has a reported historical mortality rate of about 40%. It is reasonable to assume that engineered viruses might possess a combination of attributes that allow them to spread widely with high virulence, making an outbreak potentially extremely deadly and disruptive. The Rapid Deployment Vaccine Collaborative (Radvac) is actively preparing to mitigate the effects of both natural and human-designed outbreaks—including those that have the potential to severely impact or shut down normal supply chains and basic infrastructure.

Radvac was founded in the earliest days of the SARS-CoV-2 pandemic for the rapid deployment of experimental vaccines, and recently has expanded its countermeasure initiatives to anticipate and defend against novel pathogens for which no countermeasures currently exist. Today, Radvac has research programs in multiple areas of rapidly deployable pathogen countermeasures, including vaccines, antivirals, and broad-spectrum antiviral nasal sprays. Given these focus areas employing cutting-edge technologies, it might be surprising that Radvac is also committed to bringing back and modernizing one of the oldest and most historically important pathogen countermeasures.

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https://www.nytimes.com/2026/04/29/us/ai-chatbots-biological-weapons.html?unlocked_article_code=1.elA.yXfD.VWpwsS4TfZBN&smid=url-share

² [Building an early warning system for LLM-aided biological threat creation | OpenAI](#)

³ [Opportunities to Strengthen U.S. Biosecurity from AI-Enabled Bioterrorism: What Policymakers Should Know](#)

The variolation innovation

Widespread use of vaccines is relatively new, but modern vaccines were preceded by centuries of progressively less dangerous precursors. In fact, one approach—a technique called *variolation*—was not only the first recorded method used to prevent or reduce severity of a disease (smallpox) by deliberately infecting people with an attenuated form of the pathogen, the practice was the forerunner of modern vaccination.

The variolation procedure involved exposing a person to material taken from a smallpox patient (or recently variolated person), with the hope that the resulting infection would be mild yet provide robust immunity to the virus. Often, this was done through a process called inoculation—by rubbing smallpox scabs or fluid from pustules into scratches or incisions in the skin of the recipient. While the smallpox virus was normally spread through the air and caused a severe infection starting in the lungs, infection by inoculation through the skin usually led to a milder infection that still induced a protective immune response. This is because pathogens have evolved to attack through specific routes of infection. They often infect cells through specific molecular receptors on a cell's surface. Most such receptors are absent from many other cell types. As a result, viruses have limited repertoires of attack via atypical routes. When they are placed outside of their normal “tropism” (specific cell type and tissue environments), they are often attenuated and more easily battled by the immune system.

Some of the first reported uses of variolation in China (between the 15th and 17th centuries) describe people taking smallpox scabs, grinding them up, and insufflating (or snorting) them into the nose. Since the respiratory tract is the primary smallpox infection site, one might be concerned that this approach might be likely to result in full-blown disease. This might have been one driver of reasonable attempts to give a smallpox dose via other routes, e.g. oral or dermabrasion. There must have been considerable experimental testing of a range of variations before mature techniques became more widely used, and by the 17th century, mild smallpox cases were selected as donors. It was also determined that fresh scabs were more likely to lead to a full-blown infection, so a preferred method was to use scabs that had been left to dry for days or weeks. Parts of India, Africa, and Europe developed their own variolation approaches, all of which appear to have reduced mortality rate considerably relative to natural smallpox infection.

In 1777 George Washington mandated inoculation of the U.S. Continental Army prior to their confrontation with British troops during the Revolutionary War. British

troops were often already immune to smallpox, having been exposed in crowded cities, whereas American soldiers from rural areas were largely non-immune, leading to high mortality and low morale. Washington's mandatory inoculation order was a highly guarded secret to prevent the British from discovering the vulnerability of his army during recovery. Washington's decision paid off, reducing the mortality of smallpox in the army from [the prevailing 16% down to 0.33%](#).⁴

The defining advantages of modernized variolation

There are some notable advantages and defining features of “modernized variolation” (MV) that differentiate it from traditional and dangerous variolation practices of the past. Some of these come directly from modern vaccine research, including scientifically validated approaches to pathogen inactivation (prevents replication), attenuation (impairs replication), and a wide selection of adjuvants (compounds that are added to vaccines to amplify the immune response) to ensure safe but sufficient immune stimulation. Another advantageous feature of MV over traditional variolation is far deeper knowledge about a wide range of pathogens, and the specific tropism of each one, and the use of that knowledge as a procedural guide. Inoculation into a tissue that is outside of a pathogen's normal range or tropism⁵ is a form of attenuation, which we have named *extratropic attenuation*.

DEFINITION: Modernized Variolation (MV) is a form of variolation that involves some combination of pathogen inactivation (partial or complete), pathogen attenuation, and enhancement of inoculation immunogenicity by the use of one or more adjuvants. The ultimate combination of each of these options is informed by modern science and current evidence.

Ideally, both inactivation and extratropic attenuation are used together. However, on their own, inactivated (non-replicating) pathogens typically elicit weak immunity when used for mucosal immunization because only a small fraction of them typically get through initial layers of defenses, such as the highly effective mucociliary barrier that protects nasal and other mucosal tissues. If a pathogen isn't able to replicate once it does break through these defenses, it typically is rapidly cleared before it can stimulate

⁴ See page 53, Bayne-Jones, Stanhope. *The evolution of preventive medicine in the United States Army, 1607-1939*. Office of the Surgeon General, Department of the Army, 1968.

⁵ Similar to the administration of smallpox via a dermal rather than respiratory route, but informed by current scientific knowledge of the immune system and tropisms of common pathogens.

robust immunity. One certainly doesn't want to take the chance of failing to elicit a protective response—especially when dealing with a highly virulent pathogen. This is where adjuvants play a potentially critical role in MV. Most potent vaccines consist of either active but attenuated viruses, or one or more inactive immunogens plus an adjuvant. The challenge of modernizing variolation is to use the current scientific knowledge about pathogens and their tropisms, immunity, adjuvants, and vaccination to discover the right combinations of modern tools to update the historical practice of variolation.

Now that we have described the features that define MV, it is important to point out some practices with superficial similarities that we do not consider MV, and do not endorse. MV *is not* intentional infliction of completely natural infection under somewhat controlled conditions. Pathogen parties in which participants gather with an infected person to knowingly infect themselves and others⁶ are not MV, and probably will seem absurd and dangerous once people have access to scientifically validated MV approaches.⁷ In summary, the overall goal of MV is to use current science to create immunization approaches that are far safer than either natural infection or traditional variolation, and that also stimulate robust immunity.

The advantages of MV versus vaccines

Given how far vaccines have come from traditional variolation, it probably will come as a surprise to many people that MV still retains critical advantages over modern vaccination. Over the last 150+ years traditional variolation practices have been supplanted by the more convenient and safer practice of vaccination. As a result, there has been little interest in improving variolation methods. Nevertheless, the basic premise of variolation remains sound and it provides significant advantages over typical vaccination practices, including potentially low cost and immediate access at or near the site of an outbreak. There is little doubt that vaccines remain a better solution than any form of variolation. Modern vaccination practices involve controlled dosage, high immunogenicity, well-studied adjuvants⁸, preventive dosing far in advance of a pathogen

⁶ Two examples are Chickenpox parties and COVID parties.

⁷ Similarly, human challenge trials and controlled human infection models (CHIMs), often performed on troops prior to deployments, probably would not be considered MV, but there is no clear dividing line for cases in which advanced methods are used to partly inactivate or attenuate a pathogen.

⁸ Adjuvants are compounds that are added to vaccines to greatly enhance and extend the immune response.

encounter, and other attractive features. But even vaccines that receive emergency use authorization are not available or accessible when they are needed most.

Immediate access is a critical advantage for any pathogen countermeasure, especially during a serious outbreak. With the rise of the FDA and other regulatory bodies throughout the world—and the resulting complex and slow processes of clinical trialing and regulatory review—that critical speed advantage has been lost. Modern vaccines are wonderful, lifesaving inventions—as are properly functioning drug regulatory agencies—but a protracted regulatory process ensures that vaccines are not available when and where they are needed most.⁹ Pandemics caused by naturally occurring pathogens have had severe impacts on the world economy, and engineered bioweapons would likely be far worse. Countermeasures would ideally be created and deployed as quickly as possible—meaning within days or a few weeks of an outbreak. Delays will be extremely costly in terms of human misery and mortality.

Another major advantage of MV is that it should provide protection against specific variant pathogens currently in circulation. Most vaccines for common infectious diseases—e.g., all of those for influenza and SARS-CoV-2—are highly specific for the previous year's variants, but by the time the vaccines are given, those variants often have all but disappeared. Even worse, by the time many people are infected, new variants have arisen that are at least partly resistant to the vaccine. Every year such delays cause many serious illnesses and deaths, and are a main reason why influenza vaccines in the US are typically less than 50% effective. [In the past 15 years flu vaccines have only been more than 50% effective twice](#)—the same number of times they have been less than 30% effective.¹⁰

The situation with an engineered bioweapon is even more concerning. It is reasonable to expect an engineered bioweapon might be a mixture of pathogens, which would greatly complicate the countermeasure response. Imagine trying to respond to 2 or 3 different, previously unseen, virulent pathogens simultaneously. A response strategy employing standard vaccine and antiviral technologies almost certainly would be extremely slow and chaotic. In contrast, deployment of MV would allow essentially immediate response and exact matching to a pathogen—or even multiple pathogens—currently in circulation.

⁹ This is a very complex issue but this is not a criticism of the FDA. The public is largely to blame for any irrationality displayed by the FDA. It is safe to assume that the FDA cannot be made highly efficient, and that highly effective and extremely safe but novel vaccines will not be given approval when (within days of an outbreak) they are needed most.

¹⁰ <https://www.cdc.gov/flu-vaccines-work/php/effectiveness-studies/index.html>

Unlike vaccines, self-administered variolation does not require approval from government regulatory agencies; therefore, MV can be implemented very quickly during outbreaks. Furthermore, modern technologies allow the MV process to be made extremely safe, facilitating the conversion of a full-strength pathogen into an extremely attenuated version. This can even be accomplished through the same or similar means that are used in the production of modern attenuated whole virus vaccines—including the use of specific wavelengths of ultraviolet (UV) light or treatment with very low doses of common chemicals¹¹ (which can subsequently be removed or neutralized).

Another advantage of MV over typical vaccination practices is that it is much more similar to natural infection—minus the actual infection and illness. There are many unreasonable objections to vaccines and vaccination practices, but some are very reasonable and based on many decades of scientific evidence. Therefore, many of those who are hesitant or even unwilling to take certain vaccines likely will regard MV as a superior option. Notable missteps and confusing statements by public health leadership (in the US and elsewhere) have eroded confidence in vaccines. Confidence is also diminishing because of the many highly publicized flaws of vaccines. Despite the sub-optimal state of current vaccines, important vaccine research and development efforts continue to be underfunded. Furthermore, the politicization of vaccine market dynamics combined with extreme inefficiencies of vaccine regulatory approvals have reduced vaccine choice and diversity in many countries, including the US.

As one noteworthy example, today, more than six years after the start of the pandemic, there still isn't a single FDA approved mucosal vaccine against SARS-CoV-2, and none appears to be poised for approval. Furthermore, of the hundreds of SARS-CoV-2 vaccines developed and tested during the pandemic, only four were given emergency use authorization (EUA), including two highly similar mRNA vaccines: one from Moderna and another from BioNTech and Pfizer. EUA for the Johnson & Johnson vaccine was revoked by the FDA after about two years. This leaves those in the US a choice between one subunit vaccine (Nuvaxovid) and two mRNA vaccines, none of which triggers a mucosal immune response strong enough to prevent infection or

¹¹ For example, 3% hydrogen peroxide has been used to treat viruses, rendering them non-infectious and converting them into “inactivated whole virus vaccines.” Formaldehyde (at concentrations as low as 0.01%) also has been used successfully.

spread.¹² The same weakness exists for vaccines against the most serious and common respiratory viruses currently in circulation, including influenza, Respiratory Syncytial Virus (RSV), Rhinovirus/Enterovirus (significant cause of “common cold”), Human Metapneumovirus, and others. MV allows for administration in multiple ways that at least have the potential to trigger mucosal immunity.

Table 1. Summary of the advantages of Modernized Variolation (MV) over vaccines.

Category	Modernized Variolation (MV)	Conventional Vaccines
Speed of deployment	Can be deployed immediately at the site of an outbreak	Regulatory, R&D, production, and distribution delays
Specific to current variants	Uses current circulating pathogens, so it is inherently matched to these variants	Typically targets past variants; may be less effective against newly emerging strains
Regulatory requirements	Does not require regulatory approval	Requires regulatory approval, slowing availability
Cost	Potentially very low-cost and locally deployable	Often expensive due to R&D, manufacturing, and distribution
Safety	Can be made very safe using accessible inactivation tech	Generally very safe but depends on platform
Similarity to natural infection	Mimics natural infection (minus illness); broader immunity	Often induces narrow immune responses; depends on platform
Mucosal immunity	Typical administration should induce mucosal immunity	Typical vaccines (e.g., mRNA) induce weak mucosal immunity
Public acceptance (vaccine-hesitant)	May be more acceptable to individuals skeptical of conventional vaccines	Hesitancy exists due to distrust, past public health missteps, or perceived risks

¹² Although these injectable vaccines trigger strong systemic antibody responses, they elicit weak mucosal immune responses. Mucosal immunity is present at all external surfaces and orifices, and limits or prevents entry of pathogens into the body at sites of initial infection (skin, nasal and respiratory tracts, gastrointestinal tract, reproductive and urinary tracts). It is in part defined by and differentiated from systemic immunity by immunoglobulin A (IgA) antibodies and specific T-cells, whereas systemic immunity antibodies are immunoglobulin G (IgG) and a different repertoire of T-cells, although there is some overlap and coordination between these two systems.

Category	Modernized Variolation (MV)	Conventional Vaccines
Platform diversity & flexibility	Adaptable to different pathogens and delivery methods	Limited platform diversity; often dominated by a few technologies

This is not a drill

It is difficult to overstate the importance of establishing a scientifically validated MV platform as soon as possible, prior to the acceleration of increasingly severe pathogen releases that are almost certain to occur in the near future. There are multiple reasons to expect such an acceleration, but one significant driver will be AI, due to increasingly powerful, widely distributed, and open source AI models—especially those that can be run and fine-tuned locally. It is also reasonable to expect that public health infrastructures around the world will be woefully unprepared for future outbreaks, especially for an extremely virulent pathogen. And when it is time to act, the response will be unreasonably slow.

This predictable slowness of response is because concern for safety of vaccines and other countermeasures takes disproportionate precedence over speed. Historically, vaccines are at least 100 times safer than the viruses they counter, and possibly over 1000 times safer. Governments and companies understand that many people are deeply irrational and innumerate about these issues and in order to maintain trust and avoid litigation, they err disproportionately on the side of safety, which greatly slows the deployment of effective countermeasures. In contrast, MV is fairly easily understood and can be self-managed.

Other important reasons to establish effective MV practices as soon as possible are to improve both the science and the strength of the network, to become familiar with the overall process, and to get real-world experience when the risk is low. The majority of the pathogens currently in wide circulation are not lethal or immediately dangerous to most healthy people. Therefore, the present process of setting up a local network and testing out MV on oneself is not overly risky—especially if one can reasonably expect to be infected by a particular pathogen even if they do nothing, simply because the pathogen is highly prevalent in their local environment. In other words, if one is likely to become infected and ill by remaining passive and uninvolved, one is far better off learning the rationale and methods of MV, obtaining the minimal required equipment and becoming familiar with how to use it, and contributing to this critically important public good for which there currently is no reasonable substitute. When a more serious

pathogen confronts people with experience they will be in a far better position to respond effectively to keep themselves and their local communities, networks, friends, and families safe.

Establishing MV and improving the science

Variolation works; there is no reasonable doubt about that. The amount of smallpox in a few scabs is sufficient to trigger a robust immune response, and the range of concentrations of other respiratory viruses in the nasal mucus of an infected person is similar in magnitude (10^6 to 10^8 viral particles per milliliter).¹³ But viruses used in the heyday of variolation two centuries ago were often only moderately attenuated by various means. It is likely that the smallpox virus particles in scabs dried for many days or even weeks in the 17th century were mostly inactivated, although probably not completely so. Nevertheless, in the present incarnation of MV, we cannot be certain how well completely inactivated viruses will elicit immune responses. That said, given modern understandings of viruses and the immune system, there is a reasonable expectation of success with sufficient research.

There are many possible ways to administer an MV dose. Traditional approaches of intranasal insufflation, inhalation, dermabrasion, and oral administration remain valid, but today, these can be accomplished using modern equipment such as nasal sprayers, nebulizers, transdermal microneedle arrays or rollers, enteric capsules (which protect contents from the harsh environment of the stomach), and so on. Importantly, these approaches are all capable of eliciting both mucosal and systemic immunity. For dermal microneedle or dermabrasion approaches, experiments in animal models suggest that application and removal of a few layers of sticky tape at the site of administration helps remove dead skin layers and increases the effectiveness of immunization. Reusable microneedle rollers with easily sterilized titanium microneedles are widely available for commercial purchase for less than US \$20. Enteric capsules are similarly inexpensive.

There will be substantial variation in how MV triggers immunity to certain pathogens. It might turn out that several doses are required or that an optimal immune response against certain pathogens is only achieved by the inclusion of adjuvants. These and other variables need to be researched, tested, and made to work in real time, under real-world conditions.

¹³ Bernasconi, C., Katugampola, L. and Wildum, S., 2024. Comparison of influenza viral load in nasopharyngeal and midturbinate swabs. *Influenza and Other Respiratory Viruses*, 18(1), p.e13253.

Specifics

Making MV safe and effective enough to warrant a substantial community investment will require specific technologies and procedures, plus community commitments to building the necessary infrastructure for exchanging information and suitable biosamples (nasal mucus, etc.).

Technologies and procedures

The technological and procedural requirements of modernizing variolation are relatively simple and straightforward. Minimally, the following steps are required, each of which must minimize pathogen exposure and infection:

1. SAMPLE COLLECTION

- a. Donor selection
- b. Sample collection protocol
- c. Sample collection device

2. SAMPLE PREPARATION

- a. Protocol for preparing the sample for inactivation (OPTIONAL) and administration, possibly including the addition of carrier/buffer and filtration to remove unwanted material

3. PATHOGEN INACTIVATION AND/OR ATTENUATION

- a. Means for pathogen inactivation (e.g. UV 260 nm, low concentration formaldehyde, hydrogen peroxide (H₂O₂), etc.), and protocol for inactivation (inactivation can be performed before transfer to a second party, after transfer, or both)
- b. and/or means and protocol for pathogen attenuation, e.g. scientifically selected site for extratropic administration (outside of the pathogen's normal tropism)

4. ADMINISTRATION

- a. Protocol for administration
- b. Means for administration, e.g. nasal sprayer, nebulizer, microneedle array or roller, dermabrasion, oral enteric capsule, etc.
- c. Adjuvants to boost the immunogenicity of an inactivated pathogen

Ideally, each of these will be thoroughly researched and standardized. In one ideal model, most people will be inactivating pathogens themselves prior to administration. Such a model allows for non-uniformity in approaches to inactivation and administration, but even the most laissez-faire models will probably be best served by adherence to standardized protocols. Depending on the severity of symptoms, protective gear worn during a collection procedure might be as simple as a mask and gloves, or might be more elaborate.

In the following subsections, additional details are provided for each of these steps, plus others to increase the power of MV.

Sample collection and preparation

The type of sample to be collected is key to the success of the MV procedure, and this will depend on the individual pathogen. In the simple example of a respiratory virus, nasal mucus often contains the highest concentration of pathogen, especially within the first few days of symptoms. A widemouth 5 mL tube with tightly closing cap is ideal for collecting samples. A small funnel can be helpful to those inexperienced with depositing mucus from a nostril directly into the collection tube. When collecting samples from a small child, one can use a nitrile glove to gather the mucus and deposit it into a collection tube. If the person collecting is not symptomatic then a protective mask (and possibly a face shield) and gloves should be worn. Once the sample has been collected the exterior of the collection tube can be disinfected, e.g. with 70% or stronger isopropyl alcohol.

A non-diluted sample of nasal mucus is often thick and opaque. Both of these inhibit uniform pathogen inactivation, and thick consistency and presence of mucus are non-ideal for administration. For UV inactivation, it is recommended that the sample be diluted with an appropriate liquid, such as phosphate buffered saline (PBS) and filtered to remove mucus material prior to inactivation. Dilution is not necessary when using hydrogen peroxide for inactivation. For certain routes of administration the sample might be dried prior to administration, and in some cases this might be done after inactivation.

Sample sharing and the trust factor

Trust is very important to sample sharing. Unfortunately, it is safe to assume that those motivated to release a biological weapon might also be motivated to undermine trust in an open sharing MV network by various means. This could include disinformation campaigns designed to sabotage trust in the MV community networks or sharing with others samples that are tainted with toxins or poisons. An even simpler sabotage strategy would be to share samples of healthy people, thereby defeating the

immunization potential of the MV approach. In scenarios involving highly virulent pathogens, such efforts might prove deadly. The easiest way for an individual to solve the problem of sample safety is to collect or accept samples only from those they trust completely and know to be infected.

Unfortunately, this is a serious drawback for many people, and it undermines the potential power of MV by limiting the scope of the sample sharing network. Those living together often will provide the highest level of trust and sample access, but if the donor and recipient reside together then the recipient is already in an environment of extremely high exposure risk—the pathogenic barbarians are already inside the gate. This non-ideal situation sets up a race between natural infection and MV-induced protection. It is tempting to assume this is a competition between natural infection and MV-induced adaptive immunity, but MV might stimulate a few days of innate immunity, providing some time for adaptive immunity to develop. Ideally, one would be able to be inoculated well before someone in their home is ill—especially since MV, like vaccination, will require at least a week to elicit a protective adaptive immune response.

As MV matures and more people become familiar with it, there are various measures that might be taken to secure an extended sample sharing network. Nevertheless, in early incarnations of MV, most pathogens will be relatively mild and sample sharing will be determined largely by immediate access (e.g. within households and families) to samples from someone the recipient knows and trusts.

Pathogen inactivation

Inactivation of a pathogen simply means rendering it incapable of replicating. All pathogens in biological samples can be inactivated by the same means, so protocols for inactivation will vary mostly in duration or chemical concentration. However, administration site and procedures might vary substantially across different pathogens. The approach outlined in this MV document can be adapted to other pathogens, but the focus here is on the simple case of a respiratory virus, collection and inactivation of a nasal mucus sample, and administration of the inactivated inoculum.

There is substantial published literature on the inactivation of various pathogens, and there are two broad classes of approaches: physical and chemical. Physical approaches most commonly employ heat or short wavelength radiation, including gamma rays and UV (typically UV-C). Chemical approaches employ a wide range of chemistries but the most widely tested and used for vaccine research are oxidizing agents and crosslinkers. Crosslinking agents include β -propiolactone (BPL), glutaraldehyde, formaldehyde and formalin (the solution form of formaldehyde). Most

regulatory approved inactivated vaccines use formaldehyde or BPL, but relative to more modern chemical agents, they disrupt the antigenic structure of the virus, resulting in lower immunogenicity.

UV-C light of approximately 260 nm wavelength has been firmly established to damage both RNA and DNA, which is the genetic material of all known pathogens.¹⁴ A common and inexpensive UV source for irradiation is low-pressure mercury vapor lamps, which emit at a peak of 253.7 nm. Irradiation times for inactivating most pathogens are well established. Adenovirus has a double-stranded DNA genome and is well known to require a high dose of UV irradiation, but this is relatively quickly and easily achieved with commercially available lamps.¹⁵ Nasal mucus from an infected person is often opaque and published research shows that UV of about 260 nm has low penetration of biological material. Therefore, the sample needs to be diluted and clarified prior to irradiation. Although suitable lamps are available, there is no simple device designed explicitly for the purpose outlined herein. Therefore, Radvac has researched and developed prototype devices for UV sample inactivation, and we have investigated options for the design and manufacturing of a device made specifically for MV. However, after research and testing of UV, we have concluded that there are superior chemical options that are simpler to implement.

Recent developments in virus inactivation research show that there are promising methods employing commonly available chemicals that are simple, effective, and inexpensive. Widely available hydrogen peroxide (H₂O₂) solutions are typically 3%, and this concentration has been shown to rapidly and completely inactivate viral pathogens while partially retaining antigenic structures.¹⁶ All other pathogens are also susceptible to killing or inactivation by hydrogen peroxide. Nasal mucus contains cells that produce catalases and peroxidases that inactivate peroxide, but this effect should not be a problem during sample treatment by the concentrations and volumes of hydrogen peroxide described herein. Peroxides also require metal catalysts (e.g. copper or iron) to efficiently damage nucleic acid genomes. Copper aids in olfaction (sense of smell)

¹⁴ Except for prions, which typically only reside in the neural tissue of infected animals.

¹⁵ One reason for this is because the complementary strand enables efficient repair of the damaged strand by host DNA repair systems. Ideally, a pathogen is irradiated with sufficient energy to induce enough damage that DNA repair systems cannot repair the damage.

¹⁶ A recent variation of this approach uses much lower concentrations of hydrogen peroxide in combination with copper and methisazone, better preserving antigenic structures, resulting in higher antibody titers. Amanna, I.J., Thomas, A., Engelmann, F., Hammarlund, E., Raué, H.P., Bailey, A.L., Poore, E.A., Quintel, B.K., Lewis, A.D., Axthelm, M.K. and Johnson, A.L., 2024. Development of a hydrogen peroxide-inactivated vaccine that protects against viscerotropic yellow fever in a non-human primate model. *Cell Reports Medicine*, 5(7).

and is therefore [found in human nasal mucus](#) at a relatively high concentration of about 16 µg/dL, which is about 50% higher than the concentration found in blood.¹⁷

Because this approach employs safe, inexpensive, and widely available hydrogen peroxide, which completely inactivates pathogens but at least partly preserves antigenic structure, it is the primary focus of current Radvac inactivation research on MV.

Pathogen attenuation

In contrast to pathogen inactivation, attenuation means that a pathogen remains able to replicate, but more slowly and inefficiently. Relative to complete inactivation, attenuated replication can elicit a much stronger immune response. Certain modern attenuation approaches recode the genome of a virus to greatly slow its replication, or selectively passage it to render it capable of replication only in colder tissues, like the nose, but incapable of replicating at higher core body temperature. The first approach, *codon deoptimization*, substitutes rare or suboptimal tRNA codons for common ones in the viral genome, thereby [impairing replication via multiple mechanisms](#).¹⁸ There are fewer strategies available for attenuating a pathogen to be used directly for inoculation. Nevertheless, the success of extratropic inoculation for smallpox was an important proof of principle, and there are others—some of which probably remain forgotten or undiscovered because the science of variolation has been neglected for so long.

Given what is known about the approaches described above for completely inactivating pathogens, it is reasonable to assume that milder versions—such as short duration or low concentration hydrogen peroxide treatment—also have the potential to attenuate them.

The majority of viruses responsible for the most common outbreaks, and those that are the most deadly, have RNA genomes. These include Ebola, Marburg, Rabies, HIV, Hepatitis C, coronaviruses, and Influenza. Treatment of an RNA virus with hydrogen peroxide inflicts oxidative damage on bases of the genomic RNA. The most susceptible RNA base is guanosine, which is oxidized to 8-hydroxyguanosine (8-OHG). [When the host cell ribosome encounters 8-OHG it stalls](#), reducing the speed of replication, but not entirely stopping it, if the number of 8-OHG in the genome isn't large.¹⁹

¹⁷ Block, E., Batista, V.S., Matsunami, H., Zhuang, H. and Ahmed, L., 2017. The role of metals in mammalian olfaction of low molecular weight organosulfur compounds. *Natural product reports*, 34(5), pp.529-557.

¹⁸ Groenke, Nicole, et al. "Mechanism of virus attenuation by codon pair deoptimization." *Cell reports* 31.4 (2020).

¹⁹ Kong, Qiongman, and Chien-liang Glenn Lin. "Oxidative damage to RNA: mechanisms, consequences, and diseases." *Cellular and Molecular Life Sciences* 67.11 (2010): 1817-1829.

Routinely encountered DNA viruses include typically mild and very widespread viruses such as “common cold” Adenoviruses; but DNA viruses also include Poxviruses (smallpox, Mpox), Herpes, HPV, Polyoma viruses, and Hepatitis B. Similar to guanosine in RNA, guanine bases in DNA viruses are particularly susceptible to oxidation. Conversion of guanine to 8-hydroxy-2'-deoxyguanosine (8-OHdG) in DNA causes mispairing during replication and can lead to mutation or mistranslation. However, unlike RNA, oxidatively damaged DNA is repaired by cellular enzymes. Therefore, somewhat stronger treatment with hydrogen peroxide probably is needed for attenuation, and it often isn't obvious whether an infection is due to an RNA or DNA virus—or another pathogen type altogether, e.g. a bacterium. Nevertheless, partial inactivation as an attenuation technique is an area of active investigation and holds great promise.

And this approach might be combined with extratropic attenuation. For example, nasal mucus containing a respiratory pathogen might be treated with a mild dose of hydrogen peroxide and then inoculated into the skin or taken orally in an enteric capsule. These relatively straightforward means of attenuating an existing pathogen are focus areas of our MV research.

Administration

Once the sample is prepared and inactivated it can be self-administered by various means including the following: nasal sprayer or nebulizer into the nostrils (or lungs); transcutaneously (topical application of vaccines onto the skin, e.g. using patches or creams) or transdermally (use of microneedles to pass through the stratum corneum (the outermost skin layer) to reach the deeper dermal layers); or orally. Radvac currently is experimenting with two oral approaches: dental floss and enteric capsules, e.g. nasal mucus is loaded into an enteric capsule and swallowed (this strategy aligns well with other Radvac projects on yeast expressing immunogens). Other routes and methods of administration are possible but these are the simplest, and required delivery technologies are commercially available.

Adjuvants

Fully inactivated viral pathogens typically have low immunogenicity but would be ideal for immunization if their immunogenicity could be amplified. There are multiple means for increasing immunogenicity including repeated dosing (which can be relatively easy and is only limited by sample availability) or the use of an adjuvant. Fortunately, if the sample used for self-inoculation is infected nasal mucus (or similar infectious material), then it already contains elevated concentrations of many immunostimulants, including multiple interferons (IFNs), including Type I (IFN- α and IFN- β), Type II (IFN- γ),

and Type III (IFN- λ) interferons; pro-inflammatory cytokines including Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF- α); chemokines like IL-8 that attract white blood cells (like neutrophils and macrophages) to the site of infection; and alarmins that trigger early immune responses, e.g., IL-33.

In addition to these immunostimulants produced by the human body, many experimental adjuvants have been shown to boost immunogenicity, and some of these are readily available, depending on one's location. The innate immune system has evolved to recognize as foreign a range of microbial structures, such as lipids and polymers. Evolutionarily conserved molecular signatures, such as PAMPs (Pathogen-Associated Molecular Patterns), trigger strong immune responses by binding to host toll-like receptors (TLRs).

The antibiotic Neomycin, an aminoglycoside antibiotic naturally produced by the soil bacterium *Streptomyces fradiae*, can be used as an adjuvant for both dermal and intranasal administration approaches. It isn't an FDA-approved adjuvant, but it has been shown to potently induce the expression of interferon-stimulated genes, and TLR pathways in a manner similar to other adjuvants.^{20, 21} Neomycin sulfate is present at a concentration of 3.5 mg/g in Neosporin or generic triple or double antibiotic ointment or cream. Triple antibiotic creams also contain two other antibiotics: bacitracin zinc and polymyxin B sulfate, and some double antibiotic creams (e.g. Neosporin + pain relief, which also contains the topical anesthetic pramoxine) contain neomycin sulfate and polymyxin B sulfate. Both bacitracin and polymyxin B have shown efficacy as mucosal adjuvants (although the data for bacitracin are limited).^{22, 23} Therefore, double and triple antibiotic creams are not only excellent for their antibacterial properties for intranasal and dermal administration approaches, they also serve as effective adjuvants, stimulating both mucosal and systemic immunity when administered together with an immunogen.

Other microbial polymers useful as adjuvants are immunostimulatory β -glucans (e.g. 1,3 and 1,6 linkages), which are produced by many yeasts including *Saccharomyces*

²⁰ Mao, T., Kim, J., Peña-Hernández, M.A., Valle, G., Moriyama, M., Luyten, S., Ott, I.M., Gomez-Calvo, M.L., Gehlhausen, J.R., Baker, E. and Israelow, B., 2024. Intranasal neomycin evokes broad-spectrum antiviral immunity in the upper respiratory tract. *Proceedings of the National Academy of Sciences*, 121(18), p.e2319566121.

²¹ Cohen, J.I., 2018. New Activities for Old Aminoglycosides. *Nature microbiology*, 3(5), p.531.

²² Raehs, Susanne C., et al. "The adjuvant effect of bacitracin on nasal absorption of gonadorelin and buserelin in rats." *Pharmaceutical research* 5.11 (1988): 689-693.

²³ Gulani, Mahek, et al. "Evaluation of Polymyxin B as a Novel Vaccine Adjuvant and Its Immunological Comparison with FDA-Approved Adjuvants." *Vaccines* 13.12 (2025): 1232.

cerevisiae, a yeast that is widely available and commonly used to make bread. Purified yeast polysaccharides are also commercially available, often as nutritional supplements. Product labels should say yeast derived, and specify both 1,3 and 1,6 β -glucans. These are ideal for oral uses since they are specifically produced and sold for human consumption. These purified polysaccharides can also be used as intranasal or oral adjuvants.

Alum is the longest and most widely used adjuvant and it is widely available. It isn't actually a single chemical entity but multiple forms of aluminum salts—most commonly aluminum hydroxide, aluminum phosphate, or potassium aluminum sulfate. Calcium phosphate is used in an analogous manner. Calcium phosphate gels are useful for transcutaneous or transdermal vaccination. Imiquimod is a potent adjuvant for transcutaneous, transdermal, or nasal administration, and is available as a prescription skin cream (Aldara, 5% imiquimod, Bausch Health; Aldara can be applied topically on the outside of the nose for intranasal administration, or it can be applied to the skin for dermal inoculation). Chitosan, a material Radvac has used to produce vaccine nanoparticles, has been used in many research studies and preclinical trials as a general purpose mucosal adjuvant.²⁴

A newly identified and promising mucosal adjuvant is carbomer (carboxyvinyl polymer), a very common ingredient in cosmetics, personal care products, and pharmaceuticals.²⁵ It is a type of carboxy vinyl polymer (CVP), a high-molecular-weight, cross-linked polyacrylic acid polymer. Carbomer is inexpensive and widely available for purchase. Typical of synthetic polymers, there are many varieties of carbomer with a range of physical properties, especially with respect to gel formation and viscosity. Sasaki and colleagues showed that crosslinking of carbomer is essential for adjuvant activity, and that low to moderate viscosity carbomer appears to work as well as high viscosity, and is easier to work with at higher concentrations.

Other adjuvants for consideration are certain microbially produced polysaccharides that stimulate innate immune responses. One example is curdlan, a natural, beta-glucan (unbranched-1,3-glucan) produced by certain bacteria. It is used in the food industry as a gelling and stabilizing agent, and is widely available for purchase. Certain adjuvants might be used to pre-treat an inoculation site, while others might be more appropriate for co-administration with the pathogen. For example, topical

²⁴ [Chitosan-based mucosal adjuvants: Sunrise on the ocean - PMC](#)

²⁵ Sasaki, E., Asanuma, H., Momose, H., Maeyama, J.I., Moriyama, S., Nagata, N., Suzuki, T., Hamaguchi, I., Hasegawa, H. and Takahashi, Y., 2025. Calboxyvinyl [sic] polymer adjuvant enhances respiratory IgA responses through mucosal and systemic administration. *npj Vaccines*, 10(1), p.28.

Aldara/imiquimod is a good candidate for dermal preparation because it triggers potent innate immunity that lasts for days when rubbed on the skin. Double antibiotic cream containing a topical anesthetic might be ideal to apply to the skin immediately prior to the use of a microneedle roller. Other promising mucosal adjuvants are being researched very actively. Radvac is eager to identify and test these candidates. Please let the core team know if you have information on this front.

Summary table of adjuvants

Adjuvant	Potential route	Availability / formulation	Source / potential role
Neomycin	Topical, intranasal, floss	Neomycin sulfate is present in many topical antibiotic preparations	Microbial antibiotic; innate immune stimulation
Polymyxin B	Topical, intranasal, floss	Commonly combined with neomycin in double/triple antibiotics	Microbial antibiotic; innate immune stimulation
β-glucans (1,3/1,6)	Topical, oral, intranasal, floss	Widely available; GRAS, safe for human consumption	Fungal (yeast) polysaccharide; innate immune stimulation
Alum	Formulation-dependent	Widely available; not a single chemical entity	Established systemic vaccine adjuvant
Imiquimod	Topical; transdermal, nasal (external)	Prescription 5% cream (Aldara)	Dermal pre-treatment
Chitosan	Topical, oral, intranasal, floss	Widely available; used in research and biomaterials	Mucosal adjuvant and vaccine-delivery material
Carbomer (carboxyvinyl polymer)	Topical, oral, intranasal, floss	Very common in a range of products; inexpensive	Promising newer mucosal adjuvant
Curdlan	Topical, oral, intranasal, floss	Widely used as a food gelling/stabilizing agent	Microbial polysaccharide; innate immune stimulation

Infrastructure for exchanging information and samples

Initial uses of MV have been inside the home, among family members. Therefore, community and organization-scale entities are not necessary for effective implementation of MV. Nevertheless, for the most effective defense, community involvement will be important for establishing a reliable defense against an emerging pathogen. The following are some key elements and considerations. All are topics for improvement.

- An online, fault tolerant bellwether/sentinel system that notifies people in a given location when an outbreak is occurring nearby, or is likely to occur soon according to historical transmission patterns.
 - App or website
- A sample sharing infrastructure, including:
 - Network for sample sharing / distribution
 - Initially, localized, well-known and trusted sources
 - Informed consent documents and protocols for participation
 - Protocol(s) for sample sharing / distribution
 - Locations for sample collection
 - Locations for sample dropoff and storage

Tools for reporting and measuring side effects, adverse events, and correlates of protection

- Online reporting tool
 - General descriptions of sample donor and recipient
 - Are the two in frequent close contact?
 - Do they live together or apart?
 - How long had donor been symptomatic at collection time?
 - Type of sample? (nasal mucus, saliva, other?)
 - Other?
 - General type of illness (respiratory, GI, other?)
 - Symptoms

- Duration
- Outcomes
- Antibody measurements*
 - IgG, possibly subtypes
 - IgA, possibly subtypes
- Neutralizing antibody titer; plasma neutralization assay; pseudovirus neutralization assay*
- T-cell response characterization; ELISpot*
- Others?

*These are only possible if the pathogen is identified. This can be done with point-of-care testing for certain viruses like influenza and SARS-CoV-2, and there are lab tests for the identification of other common pathogens, e.g., RSV, but certain other pathogens will likely need to have their genomes sequenced.

One critically important determinant of the success of the MV network is sample access and sharing. Of course, individuals within the larger Radvac network can establish their own local collection points and networks by organizing neighbors, friends, and family. In order to make their local network more effective they might work with organizations that are likely sources for index cases for newly arising pathogen variants. Possible collection sites include places where sick people are highly concentrated, e.g., healthcare centers and clinics, doctors offices, schools, daycare facilities, etc. Locations for dropoff and storage can range from individual homes to facilities specially established for these activities. Some of these locations might include distribution of protective gear, collection devices, and so forth.

How you can help

All of these key elements are in the formative stages. Please send us feedback and suggestions to improve and build out the urgently needed infrastructure to defend against a next pandemic. Thank you for participating in the Radvac community and thanks in advance for contributing to this important cause.

PROTOCOLS

The first of the following set of protocols describes sample collection. Subsequent protocols describe administration of the sample collected in the first protocol.

General considerations

- Four different inoculation approaches are listed below: intranasal, transdermal, orally administered enteric capsule, and dental floss²⁶. Always consider using intranasal administration initially. This triggers a localized innate immune response in the nasal cavity. Other approaches can be used in subsequent administrations.
- Ideally, collect and bank a sample for the possible identification of the responsible pathogen.

SAMPLE COLLECTION PROTOCOL:

This procedure involves the safe collection of an infected mucus sample. Be careful; a mistake might result in accidental infection. Ideally, this procedure is performed near a sink where the outside of the sample collection vial can be decontaminated. Be prepared to move immediately into the MV protocol.

MATERIALS:

- **Gloves**, preferably nitrile and of appropriate size so that the fingers are well fit
- **Mask:**
 - N100 or P100 strongly preferred
- Minimally N95 but great caution must be exercised.
- **Capped collection tube:**
 - Material: Polypropylene, glass, or other material inert to 3% hydrogen peroxide
 - Volume: 2 to 5 mL
 - Opening: at least 14 mm diameter
 - Graduations: optional but every 0.25 mL or 0.5 mL is ideal.
- OPTIONAL: **Small funnel** with the widest opening that still fits into the neck of the collection tube.
- OPTIONAL: **Small lab spatula:**

²⁶ Ingrole, R.S., Shakya, A.K., Joshi, G., Lee, C.H., Nesovic, L.D., Compans, R.W. and Gill, H.S., 2026. Floss-based vaccination targets the gingival sulcus for mucosal and systemic immunization. *Nature Biomedical Engineering*, 10(2), pp.370-389.

- If you are collecting a sample from a child, you might need to scrape mucus into the collection tube. It is advisable to first practice using your own mucus.

PROCEDURE:

1. **Put on mask and gloves.**
2. **Open the bottle of 70% isopropyl alcohol and place near the sink.**
3. **Collect sample**, e.g. nasal mucus.
 - a. Adult source: Hold the uncapped tube under the nostril, hold the other nostril closed with a finger of the other hand, and gently expel mucus into the tube. If you have trouble accomplishing this, try using a small funnel.
 - b. Small child: collection of mucus from a small child can be challenging. First try using the funnel and collection tube; however, the mucus will tend to stick to the child's nose. In that case, this approach works reasonably well:
 - i. Uncap the collection tube and place it and the lab spatula within reach
 - ii. Bring the ends of the fingers and thumb together to form the shape of a small cup or 5 petal flower (plan to decontaminate the fingertips of the glove)
 - iii. Hold the fingertip "cup" under one of the child's nostrils
 - iv. Close the other nostril (either ask the child to use a finger to close the nostril, or use a finger of your non-collection hand to close it).
 - v. Instruct the child to blow through the nostril and grab the mucus as it emerges
 - vi. Using the small spatula, scrape the collected mucus into the collection tube
 - vii. Switch to the other nostril and repeat the collection and deposition procedure
 - viii. Tightly cap the tube.
4. **Estimate sample volume.** Either use a graduated collection tube and estimate by using the tube markings, or measure the contents using the graduated dropper or pipette.
5. **Decontamination.** Make sure the cap is screwed tightly on tube and decontaminate the outside of the tube by pouring a few milliliters of 70% isopropyl alcohol over the tube, covering the top, sides, and bottom. Turn the

tube upside down and pour a small amount of isopropyl alcohol down the sides of the tube into the cap edge. Decontaminate the glove fingertips of the collection hand.

MV Protocol version NI1.0 (Nasal insufflation 1.0)

This procedure describes a procedure for intranasal administration of a dose of a respiratory pathogen present in nasal mucus. This procedure describes pathogen inactivation, preparation, and self-administration. The first part of this procedure involves drying the sample over the course of at least 2 hours. Choose a place to dry the sample where it will not be disturbed or contaminated, avoiding excessive heat (maintain the sample at less than 95 deg F, 36 deg C) and full-spectrum UV light, e.g. sunlight. The second part of the procedure begins with self-administration of a pea-sized dose of neomycin into each nostril. This step activates expression of interferon-stimulated genes and elicits an innate immune response.

MATERIALS:

- **Gloves**, preferably nitrile and of appropriate size so that the fingers are well fit
- **Mask:**
 - N100 or P100 strongly preferred
 - Minimally N95 but great caution must be exercised.
- **Triple or double antibiotic ointment or cream (Neosporin or generic).** Active ingredients in double antibiotic: neomycin sulfate and polymyxin B sulfate. Triple antibiotic also contains bacitracin zinc. It is recommended that a tube be purchased specifically for intranasal applications, and only taken from the tube with a clean cotton swab.
- **OPTIONAL: Adjuvant**, e.g. carbomer
- **Clean cotton swabs** for applying neomycin ointment into the nostrils. Purchase a box specifically for intranasal applications.
- **3% hydrogen peroxide solution**, recently purchased (within past 2 years if kept tightly capped).
- **70% isopropyl alcohol (isopropanol).**
- **1 mL to 2 mL dropper or transfer pipette**, ideally with 0.1 mL graduation markings.
- **Small glass or ceramic plate**, ideally with a flat central area at least 4 inches or 10 cm in diameter. A clear glass plate works well, allowing you to see the thickness of the spread mixture.

- **Small lab spatula:**
 - Size: The spatula will need to fit inside the collection tube. It will be used to scrape and move mucus into and out of the collection tube, and to spread the sample onto the plate. Locate a suitable spatula for these tasks.
 - If you are collecting a sample from a child, you might need to scrape mucus into the collection tube. It is advisable to first practice using your own mucus.
- **Single-sided razor blade**
- **Clean wipe for blade**, cotton ball, tissue, etc.
- **Sterile saline nasal spray**
- **Large bore plastic straw** or rolled up piece of paper, ideally about 3 to 4 inches long.

MV PROCEDURE NI1.0:

PART ONE:

1. **Put on mask and gloves**, if they aren't already on.
2. **Add hydrogen peroxide to the sample.** Using the estimated sample volume, use the dropper to add 1 to 2 volumes of hydrogen peroxide. For example, if the mucus sample is about 0.5 mL, then add 0.5 mL to 1.0 mL hydrogen peroxide to the tube. Use 1 volume for thinner, runnier samples and 2 volumes for thicker samples.
3. **Mix.** Use the dropper or transfer pipette to mix the hydrogen peroxide with the sample. Continue mixing until the mixture appears homogeneous. The mixture should become foamy and expand in volume.
4. **Spread the mixture on the plate.** Using the spatula, remove the mixture from the tube and spread it onto the center of the ceramic plate. Cover an area of about 3 inches in diameter. You will need the spatula for this step because the foamy sample will not pour.
5. **Dry the mixture.** Place the plate somewhere where it won't be disturbed, allowing the sample to dry at a relatively slow pace (at least 2 hours for all hydrogen peroxide to decompose and evaporate).

PART TWO:

6. **A FEW HOURS PRIOR TO SELF-ADMINISTRATION: Clean out the nose and apply neomycin ointment into the nostrils.**
 - a. This step can even be done up to a day before the procedure. If you are in an infectious situation, it is ideal to self-administer neomycin daily. Blow your nose into a tissue.
 - b. Take a clean cotton swab and squirt a pea-sized dose of neomycin onto the tip of the swab, positioning most of the dose on the end of the swab.
 - c. Gently insert the swab into the nose. Depth of insertion should be determined by how deep you normally insert a swab for point-of-care testing for viral infection (e.g. COVID).
 - d. Move the swab around to spread the neomycin.
 - e. Repeat in the other nostril with another pea-sized dose of neomycin. Each nostril should receive one dose.
7. **Check sample dryness.** After a few hours the sample should be dry and the mucus film on the plate should appear as a dull, nearly transparent film. When the sample is dry essentially all of the hydrogen peroxide will have decomposed into water and oxygen gas, and the water will have evaporated from the sample.
8. **Sterilize the blade.** Take the clean, single-edged razor blade and sterilize it by moistening a cotton ball or tissue with 70% isopropyl alcohol and wiping down the blade. Be very careful not to cut yourself by wiping along the cutting edge of the blade. Make sure the blade is dry and free of alcohol.
9. **Scrape the sample off the plate.** Hold the blade at an angle to the plate surface with the blade edge facing in the direction of movement, and gently scrape the dried sample off the surface of the plate. The sample should peel off the plate in small, discontinuous, wrinkled sheets.
10. **Chop the sample into a granular powder consistency. Be very careful not to breathe on the powdered sample!** It is very light and fluffy and can be blown away very easily.
11. **Clean out the nose and hydrate the sinuses.** Blow your nose. Spray some saline into each nostril, then blow your nose again. Repeat a few times until your nose is as free as possible of mucus.
12. **Administer the sample.** Place the sample plate in front of you on a desk or table. Sit upright and place one end of the straw in one nostril. Exhale, and then bend your head down and snort half of the sample powder into the nostril, then

lift your head. **DO NOT BREATHE OUT WHEN YOUR FACE IS POSITIONED OVER THE SAMPLE!** Repeat with the other nostril.

13. **Follow with a saline chaser.** Spray saline solution once in each nostril to help moisten the dried sample.

MV Protocol version TD1.0 (Trans-dermal 1.0)

This procedure describes a procedure for transdermal administration of a dose of a respiratory pathogen present in nasal mucus. This extratropic administration is a form of attenuation. The first part of this procedure is optional and involves partial inactivation of the pathogen using hydrogen peroxide (H_2O_2). This adds an additional layer of attenuation by partly damaging some fraction of the pathogen population. It also will partly inactivate any other pathogens in the sample, which is an important consideration. The second part of the procedure begins with preparation of the selected dermal surface. Choose a surface that is easy to access and sufficiently large for the dermal roller. The size of the dermal surface in this protocol is 3 inches long by 2 inches wide, and afterward will be covered by a 4 inch by 3 inch bandage.

MATERIALS:

- **Gloves**, preferably nitrile and of appropriate size so that the fingers are well fit
- **Mask:**
 - N100 or P100 strongly preferred
 - Minimally N95 but great caution must be exercised.
- **1 mL to 2 mL dropper or transfer pipette**, ideally with 0.1 mL graduation markings.
- **Dermal microneedle roller**, generally with tungsten needles, 0.5 mm to 1.0 mm in length (e.g., 540 needles). Needles of this length typically do not hurt or draw blood, but penetrate to the deeper layers of the epidermis, reaching some immune cells. Needles 1mm in length are able to reach the dermis, and a greater range of immune cells; they might hurt a bit and draw blood but light pressure can be used to limit penetration. Reusable microneedle rollers with easily sterilized titanium microneedles are widely available for commercial purchase for less than US \$20.
- **Shallow plastic tray or trough for sterilizing the microneedle roller.** The tray should hold a few millimeters depth of isopropyl alcohol, allowing the microneedle roller to be placed in the tray and gently rolled to sterilize all

microneedles and the roller surface. Some microneedle rollers come in a plastic case that can be used as the tray (e.g., Cletina brand, available on Amazon).

- **Sticky medical or surgical tape.** For removing layers of dead skin from the area selected for administration. Experiment with tape in advance of the procedure to ensure that tape is sticky and somewhat difficult to remove. This ensures that all dead skin will be removed. Other sticky tape (e.g., duct tape) also works but sterility is important.
- **Triple or double antibiotic ointment or cream (Neosporin or generic).** Active ingredients in double antibiotic: neomycin sulfate and polymyxin B sulfate. Triple antibiotic also contains bacitracin zinc. It is recommended that a tube be purchased specifically for intranasal applications, and only taken from the tube with a clean cotton swab.
- **Clean cotton swabs** for applying neomycin ointment onto the skin. Purchase a box specifically for such applications.
- **70% isopropyl alcohol.**
- **Cotton balls.**
- **Small lab spatula:**
 - Size: The spatula will need to fit inside the collection tube. It will be used to scrape and move mucus into and out of the collection tube.
- **Bandage, 4 x 3 inches.**
- **OPTIONAL: razor** for shaving the area prior to use of tape.
- **OPTIONAL: Adjuvant**, e.g. Aldara (imiquimod) cream, carbomer, etc.
- **OPTIONAL: 1 mL to 2 mL dropper or transfer pipette**, ideally with 0.1 mL graduation markings.
- **OPTIONAL: Small funnel** with the widest opening that still fits into the neck of the collection tube.
- **OPTIONAL: 3% hydrogen peroxide solution**, recently purchased (within past 2 years if kept tightly capped).

MV PROCEDURE TD1.0:

If you plan to employ the optional partial inactivation, be prepared to move directly into Part Two of the protocol. Because the first steps of Part Two take several minutes, plan to start Part Two even before the end of the incubation period of Part One. If you plan on pre-applying an adjuvant, do this in advance of the procedure (see the initial step of the procedure below).

PART ONE (OPTIONAL):

1. **OPTIONAL: Apply adjuvant to the dermal surface.** 1 to 36 hours prior to the sample administration, apply Aldara cream, double antibiotic creams (e.g. Neosporin + pain relief, which contains the topical anesthetic pramoxine), or other adjuvant to the target area of the skin.
2. **Put on mask and gloves**, if they aren't already on.
3. **Add hydrogen peroxide.** Using the estimated sample volume, add an equal volume of hydrogen peroxide. For example, if the mucus sample is about 0.5 mL, then add 0.5 mL hydrogen peroxide to the tube.
4. **Mix.** Use the dropper or transfer pipette to mix the hydrogen peroxide with the sample. Continue mixing until the mixture appears homogeneous. The mixture should become foamy and expand in volume.
5. **Partially inactivate pathogen.** Allow the mixture to incubate at room temperature for 15 to 30 minutes. This step partially attenuates the pathogen by partial inactivation. You can increase the incubation time to achieve greater inactivation, or decrease incubation time to allow greater pathogen activity.

PART TWO

6. **Select and mark out a patch of skin.** A good size is 2 inches by 3 inches (about 5 x 7.5 cm), and 3 x 4 inch bandages are a good size to cover this area. In general, the upper part of the thigh (top of the thigh when sitting down) is the easiest large surface to work on. Plus, this is in close proximity to multiple lymph nodes in the pelvis and groin area. You can mark the area by placing dots at each corner of the rectangle with a marker.
7. **Sterilize the microneedle roller.**
 - a. Pour isopropyl alcohol into the tray to a depth of a few millimeters.
 - b. Place the microneedle roller in the tray and gently roll it to sterilize all microneedles and the roller surface.
 - c. Shake the excess isopropyl alcohol off the roller.
 - d. After the roller is sterilized, prop it on the edge of the tray to allow the needles to dry before use.
8. **Shave the dermal surface if it isn't already free of hair.** Ideally, shave at least an inch outside the boundaries of the target patch so that the bandage does not stick to hair.
9. **Preparation of dermal surface by tape adhesion and removal.** This step should be done immediately prior to the administration procedure. Initially, the

tape will remove layers of dead skin and the tape will no longer stick well to the skin. Each successive piece of tape will stick better than the last. Repeat until all dead skin is removed.

- a. Take a piece of tape about 4 to 5 inches long to cover the full length of the roller area plus an inch on each end to hold. Apply it to skin surface and press down to ensure it is completely adhered. Fully cover the 2 x 3 inch administration area. Remove the tape. Restick the same tape and remove. This might take multiple side-by-side applications of the tape to cover the full width of the administration area.
- b. Repeat this procedure until the tape is sticking securely to the skin (about five to ten pieces of tape, in total).

10. Clean and sterilize the dermal surface.

- a. Wet a cotton ball with 70% isopropyl alcohol and thoroughly clean the surface of the skin area selected for administration.
- b. Allow the surface to dry before moving to the next step.

11. Apply and spread the sample on the dermal surface.

- a. Use the dropper or transfer pipette to remove the sample from the tube and apply it to the dermal surface.
- b. Use the end of the dropper to spread the sample. Alternatively, sterilize the spatula using a cotton ball and 70% isopropyl alcohol, wave the spatula in the air briefly to dry all of the alcohol, and then spread the sample with the spatula. Fully cover the administration area.

12. Roll the covered dermal area with the microneedle roller. Roll the sample into the skin by moving the roller over the target area 10 to 20 times.

13. Apply triple antibiotic ointment. Use a sterile gloved finger or the spatula to apply triple antibiotic ointment in a thin layer to the entire administered area.

14. Apply a bandage. Use a 3 inch by 4 inch bandage to cover the administration area.

MV Protocol version EC1.0 (Enteric Capsule 1.0)

This procedure describes a procedure for oral administration of a dose of a respiratory pathogen present in nasal mucus. This procedure involves drying the sample over the course of at least 2 hours. Choose a place to dry the sample where it will not be

disturbed or contaminated, avoiding excessive heat (maintain the sample at less than 95 deg F, 36 deg C) and full-spectrum UV light, e.g. sunlight.

Do not eat for at least 6 hours prior to the procedure.

MATERIALS:

- **Gloves**, preferably nitrile and of appropriate size so that the fingers are well fit
- **Mask:**
 - N100 or P100 strongly preferred
 - Minimally N95 but great caution must be exercised.
- **OPTIONAL: 3% hydrogen peroxide solution**, recently purchased (within past 2 years if kept tightly capped).
- **OPTIONAL: Adjuvant**, e.g. carbomer
- **1 mL to 2 mL dropper or transfer pipette**, ideally with 0.1 mL graduation markings.
- **Small ceramic plate**, ideally with a flat central area at least 4 inches or 10 cm in diameter.
- **Single-sided razor blade**
- **Clean wipe for blade**, cotton ball, tissue, etc.
- **Enteric capsules**, e.g., size 00.
- **Capsule fill kit**, with funnel, capsule holder, tamp, and spatula.

MV PROCEDURE EC1.0:

1. **Put on mask and gloves**, if they aren't already on.
2. **OPTIONAL: Using Part One of MV Protocol version NI, add hydrogen peroxide to the sample to partly or fully inactivate the pathogen.** Using the estimated sample volume, use the dropper to add an equal volume of hydrogen peroxide. For example, if the mucus sample is about 0.5 mL, then add 0.5 mL hydrogen peroxide to the tube. Use the dropper to mix the hydrogen peroxide with the sample.
3. **Spread the sample on the plate.** Using the dropper or spatula, remove the sample from the collection tube and spread it onto the center of the ceramic plate. Cover an area of about 3 inches in diameter. You might need the spatula for this step due to sample viscosity.
4. **Dry the mixture.** Place the plate somewhere where it won't be disturbed, allowing the sample to dry at a relatively slow pace (at least 2 hours for all liquid to evaporate).

5. **Check sample dryness.** After a few hours the sample should be dry and the mucus film on the plate should appear as a dull, nearly transparent film.
6. **Sterilize the blade.** Take the clean, single-edged razor blade and sterilize it by moistening a cotton ball or tissue with 70% isopropyl alcohol and wiping down the blade. Be very careful not to cut yourself by wiping along the cutting edge of the blade. Make sure the blade is dry and free of alcohol.
7. **Scrape the sample off the plate.** Hold the blade at an angle to the plate surface with the blade edge facing in the direction of movement, and gently scrape the dried sample off the surface of the plate. The sample should peel off the plate in small, discontinuous, wrinkled sheets.
8. **Chop the sample into a granular powder consistency.** Keep your mask on during this step.
9. **OPTIONAL: mix adjuvant(s) with the sample,** e.g., add to the powdered sample about $\frac{1}{4}$ volume (of the estimated volume of the sample powder) of carbomer and/or dried yeast (both can be used, as can other adjuvants).
10. **Fill a capsule.**
 - a. Place the larger section of a 00 enteric capsule in a capsule holder and place a funnel into the capsule.
 - b. Use a small spatula to scrape the dried sample into the capsule.
 - c. Use a capsule tamp to tamp down and compact the sample into the capsule. Continue until the capsule is full or all of the sample has been placed in the capsule.
 - d. Remove the funnel and place the unfilled side onto the filled side to close the capsule. If sample is left over, fill additional capsules.
11. **Sterilize the exterior of the capsule.** Very briefly wipe down the capsule with a cotton swab dipped in 70% isopropyl alcohol, and allow the capsule to dry.
12. **Remove mask and consume the capsule(s).** Use a small amount of water to consume the capsule(s) (75 to 100 mL, or about 3 ounces). Do not eat for an additional 2 hours after self-administering the capsule.

MV Protocol version DF1.0 (Dental Floss 1.0)

This procedure describes a procedure for oral cavity administration of a dose of a respiratory pathogen present in nasal mucus by the use of dental floss. This procedure describes pathogen inactivation, preparation, and self-administration. This extratropic

administration is a form of attenuation. The first part of this procedure involves partial inactivation of the pathogen by mixing it with hydrogen peroxide (H_2O_2). This adds an additional layer of attenuation by partly damaging some fraction of the pathogen population. It also will partly inactivate any other pathogens in the sample and oral cavity. The second part of the procedure begins with self-administration of the mixture into the oral cavity followed by flossing. This step activates expression of interferon-stimulated genes and elicits an innate immune response.

MATERIALS:

- **Gloves**, preferably nitrile and of appropriate size so that the fingers are well fit
- **Mask:**
 - N100 or P100 strongly preferred
 - Minimally N95 but great caution must be exercised.
- **OPTIONAL: Adjuvant**, e.g. yeast beta-glucans, carbomer
- **3% hydrogen peroxide solution**, recently purchased (within past 2 years if kept tightly capped).
- **1 mL to 2 mL dropper or transfer pipette**, ideally with 0.1 mL graduation markings.
- **OPTIONAL: Small lab spatula**
 - Size: The spatula will need to fit inside the collection tube. It will be used to scrape and move mucus into and out of the collection tube. Locate a suitable spatula for these tasks.
 - If you are collecting a sample from a child, you might need to scrape mucus into the collection tube. It is advisable to first practice using your own mucus.

MV PROCEDURE DF1.0:

PART ONE:

14. **OPTIONAL: Put on gloves.**
15. **Add hydrogen peroxide to the sample.** Using the estimated sample volume, use the dropper to add two volumes of hydrogen peroxide. For example, if the mucus sample is about 0.5 mL, then add 1.0 mL hydrogen peroxide to the tube.
16. **Mix.** Use the dropper or transfer pipette to mix the hydrogen peroxide with the sample. Continue mixing until the mixture appears homogeneous. The mixture should become foamy and expand in volume.

17. **Incubate.** Let the mixture sit for 15 to 30 minutes at room temperature to partially inactivate the pathogen.

PART TWO:

18. **Pre-floss and rinse the oral cavity.** Prior to placing the sample into the oral cavity, carefully floss between all teeth. Make sure to floss below the gumline. Rinse with a mouthful of clean water.
19. **Administer the sample.** Using the dropper, transfer pipette, or spatula, place the mixture into the oral cavity. Ideally, place the mixture onto the gumline. DO NOT SWALLOW THE MIXTURE!
20. **Floss the oral cavity again BUT DO NOT RINSE AFTERWARD.** Carefully floss between all teeth, making sure to work the mixture into the space between teeth and gum. Make sure to floss below the gumline. DO NOT RINSE AFTER FLOSSING. Spit out the excess mixture or swallow it. Do not eat or drink anything for at least 30 minutes.

APPENDIX: Case studies

Case 1; April 29 to May 10, 2026

Case subject: Preston Estep, Radvac founder and Chief Scientist

Case topic: This case study is a first-person narrative describing the development and initial use of MV. This study is presented as an anecdote, not as evidence of the effectiveness of the procedure.

Case report narrative: The full narrative of the case study is published [here](#) on Substack.

Summary: A protocol was developed and tested for the collection of a nasal mucus sample from an infected donor. Two separate protocols for inactivation of the pathogen within the sample were tested: 1) UV-C irradiation, and 2) hydrogen peroxide treatment. In each of these, after the sample was inactivated it was administered intranasally. I did not use antibacterial adjuvant ointment either before or after administration because I wanted to test the MV approach minus a potent stimulator of innate immunity, like neomycin. However, I am including double or triple antibacterial administration in the pilot protocol.

Outcomes: In late March I tested the UV-C protocol. Subsequent to UV-C irradiation and sample administration, I became ill with a respiratory infection. I don't report the timeline in detail below because I became ill soon after using the protocol. I did not attempt the UV-C inactivation protocol again because it was so complicated, required the construction of a device, and did not work. Therefore, I moved on to testing the hydrogen peroxide approach. Subsequent to hydrogen peroxide treatment and sample administration, I did not become ill, despite members of the household showing overt signs of respiratory infection for several days. My interpretation of these results is presented at the end of the case study.

Timeline

Day 1. My brother began to snifle and clear his throat.

Day 2. My brother's symptoms worsened. My daughter's nose continued to run and she sneezed frequently. Her symptoms did not appear to worsen over the coming days.

Day 3. In the evening, my wife started showing symptoms, but they were very mild. As soon as my brother and my wife started showing symptoms, I kept my distance from them. It was still unclear whether or not our daughter was ill, but I did not have the option of keeping my distance from her. Still, when I had to lift, carry, or hug her, I held my breath (she has a habit of sneezing or coughing in my face). When I drove her in the car, I lowered the windows a few inches. I took these minimal measures to give me some infection-free time to work out the details of the MV approach.

After my UV inactivation attempt ended in failure and illness I was apprehensive about trying MV again, but the few days ahead appeared daunting. As my wife grew increasingly ill, the inside of my home would reach peak infectiousness. With this sword of Damocles hanging over me, I worked as quickly as possible to finalize the first draft of my new protocols.

Day 4. I remained free of respiratory illness symptoms the entire day.

Like my brother, my wife developed a cough, general malaise, and a runny nose that lasted about 10 days. My brother's symptoms worsened and he developed a fever. I quarantined him to the basement apartment of our house. He remained in quarantine until Day 10. I wore a mask or held my breath when I needed to go into the basement. My wife's symptoms worsened and she developed a nasty-sounding, hacking cough. I kept a distance of several feet from her while she was obviously symptomatic.

I decided that I had to take a chance on my rudimentary and untested hydrogen peroxide inactivation protocol, and my equally untested nasal self-administration protocol. I started by collecting a sample from my brother. He had been symptomatic for four days and was experiencing much more severe symptoms than my wife, so he was the best sample donor candidate. I put on gloves and a mask, grabbed a collection tube, and descended the basement stairs to pay him a visit. I uncapped and handed him the 5 mL sample collection tube.²⁷ He had some trouble depositing mucus directly from his nostrils into the 14 mm opening of the tube, but after some effort he was able to deposit about 0.5 mL into the tube. I capped the tube and immediately took it for a decontamination rinse with 70% isopropyl alcohol.

I had the tube sitting on my desk as I reviewed notes on my computer to help guide me in preparing the sample for self-administration. I had a rough protocol and some ideas for variations typed on the page, but I was in a bit of a rush because it was getting late in the evening. So I sketched an outline of the protocol in my head and gathered all the materials I thought I would need.

Due to the relative simplicity of the protocol, even the first time through went fairly smoothly—despite the fact that I was partly making up or modifying the protocol as I went. I mixed the sample with about an equal volume of 3% hydrogen peroxide, mixed it well, and spread it out onto a small, clean ceramic plate. There were a few small lumps in the sample, so I used a fork from our kitchen to homogenize the sample by mashing and mixing it on the plate, until it was spread in a fairly uniform layer about 1 to 2 mm thick. I put the plate on a table in a secure location and left the sample to dry overnight.

Day 5. I remained free of respiratory illness symptoms the entire day. I kept my distance from others in the home as much as possible until I administered the MV dose.

In the morning I retrieved the plate. The sample had dried to a dull, thin film on the plate. I used a single-edged razor blade to scrape it off the plate and to chop the sample into a granular powder.

Prior to self-administration of the inactivated sample, I cleaned out my nose by repeatedly blowing it into a tissue and alternating that with a couple squirts of saline nasal spray up each nostril. I repeated that cycle a few times. One last time I blew my nose to evacuate all contents. My left nostril was very clear and open; my right nostril remained partly closed. I rolled up a clean piece of paper into a tube a bit over 3 inches (8 cm) long and about ¼ inch (6 mm) in diameter. I snorted half the sample into the

²⁷ Graduated 5 mL polypropylene tube.

clear nostril first, then snorted the remainder into the other nostril. Time of self-administration was about 10:30 am.

I felt a mild discomfort when the sample hit the inside of my nostrils. Therefore, I decided to follow administration with a chaser squirt of sterile saline nasal spray. I went about my day and paid attention to my nose and sinuses. There was no discomfort and no obvious change in nasal health. After a few hours it seemed like the procedure was safe so I delivered a collection tube to my brother and asked him to deposit a second sample. Once again he was able to collect about half a milliliter, and I repeated the entire sample preparation procedure. This time, I dried the sample for several hours and administered the sample at about 10 pm in the evening. Time between doses: about 11.5 hours.

Day 6. I remained free of respiratory illness symptoms the entire day.

I woke up with moderate nasal congestion, which is not uncommon for me during spring allergy season. I felt mostly fine and normal throughout the day, although the nasal congestion persisted and was probably a bit heavier than usual.

Day 7. Today, nasal congestion was very heavy. I felt mentally and physically sluggish, and mildly feverish, consistent with respiratory illness symptoms. Nevertheless, I didn't feel too bad physically (70ish percent), and the weather was nice, so I spent part of the day outdoors. By the time I went to bed I still felt slightly unwell. I was increasingly certain that I had become infected.

Day 8. To my surprise, I woke up feeling better (I felt maybe 90ish percent), and felt increasingly normal as the day progressed. Nasal congestion remained moderately heavy.

Day 9. I woke up feeling normal. Nasal congestion levels returned to normal. No symptoms of respiratory illness. My brother says he is feeling better but my wife remains symptomatic, so I am not out of the woods.

Day 10. I woke up feeling normal. I remained free of respiratory illness symptoms the entire day. My brother is mostly recovered and has emerged from his quarantine. My wife remains symptomatic, so I remain cautious.

Day 11. I woke up feeling normal. I remained free of respiratory illness symptoms the entire day. My wife feels better. I assume she is not contagious. I think I have avoided infection.

Day 12. My wife is feeling well but has a lingering cough, which is normal for her. I am convinced that I have avoided infection.

My interpretation of the results

- For the UV inactivation test, it is possible that I lost the natural infection vs. MV race; or that the complexity of the UV inactivation procedure exposed me to the pathogen; or that the UV procedure wasn't fully effective and I self-administered active pathogen.
- For the hydrogen peroxide test, I administered the MV doses on the morning and evening of Day 5, and on Days 6 and 7 I thought I was becoming sick, but by Day 8 I began to feel better. It is impossible to say whether or not the MV self-administration protected me against the pathogen in our home. I was vigilant about social distancing, but I was in the same home as two (probably three) overtly sick people. I have tried social distancing within my home many times before and have failed most of the time.
- One possibility is that MV administration on Day 5 triggered strong innate immunity⁷, and that was the source of the extreme congestion and ill feelings over the following days. Maybe that alone protected me during the peak infectious period. If this is the case, adaptive immunity might have been unnecessary for protection in this situation, and the MV approach is a complicated way to achieve innate immune protection. Far easier ways exist, [including the application of triple antibiotic ointment](#) (e.g. Neosporin and generics) into the nostrils.⁸
- Nevertheless, it is also possible that I had encountered a variant of the pathogen previously and the MV procedure began to elicit adaptive memory by activating memory T cells and B cells. If the prior variant wasn't much different from the current culprit, and the past encounter was relatively recent, this response can take as little as a single day. If this is what happened, in addition to mounting an innate immune response, my body began to mount a so-called secondary adaptive response that contributed to the ill feeling and malaise I felt on Day 7.
- It is unsurprising that MV has the potential to trigger a potentially protective innate immune response, but this protection will be relatively short-lived. If a serious pathogen is in circulation and deployments of FDA approved vaccines won't occur in the foreseeable future, then it is extremely important that MV research and development lead to a protocol capable of stimulating longer-lasting adaptive immunity. Now that I have a pilot MV protocol to follow, next time there is an illness in our home I will collect a sample explicitly for the purpose of identifying the pathogen. I can assess my immune profiles before and after infection. I will bank blood and saliva samples in our lab freezer, and test and compare those to post-MV samples. I also plan to include adjuvants (double

or triple antibiotic ointment and carbomer) as a regular part of future MV dosing regimens.

Case 2; September 1-10, 2026

Case subject: Preston Estep, Radvac founder and Chief Scientist

Case topic: This case study is a first-person narrative describing the development and second use of MV. The two main highlights of this narrative are 1) this is the first time I used the transdermal protocol (MV PROCEDURE TD1.0), and 2) this the second consecutive time I have used MV in response to an illness in my home, and have avoided becoming sick.

Case report narrative: The full narrative of the case study can be found on Substack?

Summary: On Day 1 of my 4 year-old daughter showing signs of infection with a respiratory pathogen, I collected a nasal mucus sample from her. I used the hydrogen peroxide inactivation protocol for inactivation of the pathogen (add an equal volume of H₂O₂), and then this mixture was administered intranasally. Unlike the previous test in May of this year, this time I followed administration with adjuvant triple antibiotic ointment. I also used intranasal antibiotic ointment or cream every subsequent day. For the first time I also tested the transdermal administration approach using a microneedle roller to administer a second sample that was treated with an equal volume of hydrogen peroxide for 30 minutes prior to administration.

Outcomes and bottom line: This is the second time I have used Modernized Variolation protocols based on partial pathogen inactivation by hydrogen peroxide. Both times I have avoided infection with a respiratory pathogen that infected the others in my home.

Case timeline

Day 1 (Sept 1): In the morning I noticed that my 4 year old daughter was a bit cranky and her energy level was lower than normal. My wife said that she didn't seem her normal self at bedtime the night before. In the early afternoon, she began to show overt signs of a respiratory illness. Her nose was running, she was sneezing, and coughing. Soon after onset I had her blow her nose directly into a 5 mL tube. I put gloves on both hands and held the tube under one nostril at a time, closing the other nostril with a gloved finger. The mucus was very sticky and stringy and would not stay in the tube, so I used as gloved finger to gather the mucus into the tube opening. I used arm centrifuging (swing

the tube forcefully) to get the mucus into the bottom of the tube. Some of it remained adhered to the sides of the tube but I estimated the volume of mucus to be about 0.5 mL. In this protocol rough estimates are fine. I added 0.5 mL of hydrogen peroxide, mixed it well with a dropper, and spread it out onto a small, clean ceramic plate. I put the plate on a table in a secure location with good air circulation, and left the sample to dry at room temperature (about 75 deg F) for about 2 hours. The sample dried to a dull, thin film on the plate. I used a single-edged razor blade to scrape it off the plate and to chop it into a granular powder.

Intranasal administration: I first blew my nose on a tissue and then administered saline spray and then blew my nose again. I repeated this procedure. My right nostril remained mostly blocked, so I administered the majority of the sample into my left nostril by insufflating the powdered sample through a straw. I followed a couple hours later by using a clean cotton swab to apply a pea-sized dollop of triple antibiotic ointment into each nostril. Unlike the first time I used this protocol, I did not experience severe congestion, only mild to moderate congestion. Also unlike the first time, I did not feel slightly ill or feverish later or the next day.

Additional precautions: I held my breath or wore a mask when in close proximity to other people in the house, opened windows, kept HEPA air purifiers running, and ran on low speed the kitchen cooktop fan that vents outside. I ran Germ Guardian ultraviolet air sanitizers in my daughter's room and in my office and bedroom.

Day 2: I woke up feeling fine. Nasal congestion is moderate in the left nostril and still heavy in the right. In the morning I collected more of my daughter's nasal mucus (a bit less than 0.5 mL), mixed it with an equal volume of hydrogen peroxide, incubated for about 30 minutes.

Transdermal administration: I administered the mixture with a dermal roller into the top of my left thigh according to the protocol TD1.0. I applied a layer of triple antibiotic ointment to the administration area and bandaged it. The dermal roller procedure went relatively smoothly. The administration area is a bit red but no discomfort. The administration area is about 2 x 3 inches, which is covered perfectly by a 3x4 inch bandage. I used a dermal roller with 540 1.0 mm titanium needles. The pressure I used for rolling was light to moderate.

Following the transdermal administration procedure I applied a nasal dose of triple antibiotic ointment. I did this to keep my nasal mucosal immune system stimulated. I

checked the transdermal administration site later in the day and it appeared a bit blotchy and reddish but not excessively so. I continued all additional precautions.

In the evening I collected a sample and stored it for possible future identification of the pathogen.

Day 3: I feel fine. Nasal congestion is moderate in both nostrils. I decided to try a new tube of double antibiotic cream (Neosporin pain relief cream). I applied a nasal dose of the cream. The transdermal administration site appears reddish but no more than yesterday. I changed the bandage and applied a new layer of triple antibiotic ointment. I continued all additional precautions.

Day 4: I feel fine. Nasal congestion is light in the left nostril and moderate to heavy in the right nostril. I applied a nasal dose of double antibiotic cream. The transdermal administration site appears reddish but no less than yesterday. I removed the bandage. I continued all additional precautions.

Day 5: I feel fine. Nasal congestion is light to moderate moderate in both nostrils. I applied a nasal dose of double antibiotic cream. The transdermal administration site appears about normal. I continued all additional precautions.

Days 6 and 7: I feel fine. Nasal congestion is variable. My daughter and wife have mild symptoms but claim to feel much better. I discontinued wearing a mask but retained other precautions.

Days 8 and 9: My nasal passages and throat feel a bit raw. Those symptoms resolved and I attribute them to gastric reflux, which I get occasionally.

Day 10: I feel fine and my wife and daughter are fully recovered.

Outcomes: Subsequent to the administration procedures I did not become ill, despite members of the household showing overt signs of respiratory infection for several days.

My interpretation of the results

- This is the second time I have used Modernized Variolation protocols based on partial pathogen inactivation by hydrogen peroxide. Both times I have avoided infection with a respiratory pathogen that infected the others in my home. Although I cannot say for certain that the MV self-administrations protected me against the pathogen in our home, the procedures aren't terribly difficult and the outcomes have been good. I was vigilant about social distancing, but I was in the same home as two overtly sick people.
- One possibility is that MV administration triggered innate immunity and congestion in my nasal passages. Maybe that alone protected me during the peak infectious period. Over 90% of respiratory pathogens enter the body through the nasal mucosa and respiratory tract. Therefore, it makes sense to block this entry point first. After the positive outcome from my first test of this protocol I reasoned that the enhanced nasal congestion and delivery of the donor's mixture of immune signaling factors (cytokines, chemokines, and interferons) create a formidable primary defense against a respiratory pathogen. Therefore, intranasal administration will always be the first MV approach I will use against a respiratory pathogen.
- Even if innate immunity is playing a critically important role, MV has the potential to create long-lasting adaptive immunity.
- The transdermal protocol went smoothly using a dermal roller with 540 titanium microneedles, 1.0 mm long. The administration site showed signs of irritation and possible immune stimulation, but the site was not swollen and did not appear to have been infected by an adventitious pathogen. This second administration might have provided additional adaptive immunization.

Case 3: September 24-30, 2026

Case subject: Preston Estep, Radvac founder and Chief Scientist

Case topic: This case study is a first-person narrative describing the development and third use of MV. The two main highlights of this narrative are 1) this is first time I used the dental floss protocol (MV PROCEDURE DF1.0), and 2) this is the third consecutive time I have used MV in response to an illness in my home, and have avoided becoming sick.

Summary: At about 2:30pm of Day 1, my 4.5 year-old daughter showed initial signs of an illness, beginning with a mild sore throat and occasional sneezing. Later that evening

she said she felt fine but by the following afternoon her voice became hoarse, she sneezed and coughed occasionally, likely indicating she was infected with a respiratory pathogen. I collected a nasal mucus sample from her. I used the hydrogen peroxide inactivation protocol for inactivation of the pathogen (add an equal volume of H_2O_2), and then this mixture was administered intranasally. Unlike the previous tests, this time I administered double antibiotic ointment intranasally one hour prior to nasal insufflation of the mixture. I also used intranasal antibiotic ointment or cream every subsequent day. For the first time I also tested a dental floss administration approach to administer a second sample that was treated with a 2x volume of hydrogen peroxide for 30 minutes prior to administration.

Outcomes and bottom line: This is the third consecutive time I have used Modernized Variolation protocols in response to an infectious respiratory pathogen in a member of my household. All three times I have avoided infection.

Case timeline

Day 1 (Sept 24): At about 2:30pm my 4.5 year old daughter complained that her throat was a bit sore. She sneezed occasionally but it is allergy season and this is not unusual. She displayed no other overt symptoms. My wife and my brother also live in our house and they showed no symptoms.

Day 2: In the morning my daughter said she felt fine and seemed like her usual self. At about 3pm her voice became a bit hoarse and she began to show overt signs of a respiratory illness. By 6:30 pm her nose was running, she was sneezing, and coughing more frequently but symptoms were mild and she had her usual demeanor and energy.

Sample collection and inactivation: Soon after overt symptom onset I had my daughter blow her nose directly into a 5 mL tube. I held the tube under one nostril at a time, closing the other nostril with a finger. The mucus was very sticky and stringy and would not stay in the tube, so I used a finger to gather the mucus into the tube opening. I used arm centrifuging (swing the tube forcefully) to get the mucus into the bottom of the tube. Some of it remained adhered to the sides of the tube but I estimated the volume of mucus to be about 0.5 mL. Using a graduated dropper I added 0.5 mL of fresh hydrogen peroxide, mixed it well with the dropper, and spread it out onto a small, clean ceramic plate. I put the plate on a table in a secure location with good air circulation, and left the sample to dry at room temperature (about 75 deg F) for about 1

hour. After an hour the sample had dried to a dull, thin film on the plate. I used a single-edged razor blade to scrape it off the plate and to chop it into a granular powder.

Intranasal administration of adjuvant: As the sample dried, I used a gloved hand and a sterile cotton swab to administer about a pea-sized dollop of double antibiotic cream (Neosporin pain relief cream containing neomycin sulfate and polymyxin B sulfate) into each nostril. I selected this cream as a slight modification of the protocol pioneered by the Iwasaki lab at Yale, which utilizes triple antibiotic ointment. I chose the cream for this use because it is less greasy (the ointment has the consistency of vaseline). I reasoned that the greasy ointment might interfere with absorption of the mucus mixture.

Intranasal administration: I first blew my nose on a tissue and then administered saline spray and then blew my nose again. I repeated this procedure. My nostrils were both partly clear so I administered half of the sample into each nostril by insufflating the powdered sample through a straw. Time of administration was about 8:30pm. Over the next few hours I experienced moderate to heavy congestion. That night I felt pressure and discomfort in my sinuses; definite head pressure, but not quite a headache.

Additional precautions: I held my breath or wore a mask occasionally when in close proximity to other people in the house and kept HEPA air purifiers running. I ran Germ Guardian ultraviolet air sanitizers in my daughter's room and in my office and bedroom.

Other members of the house: My wife nasally administered triple antibiotic cream at about 8pm. She and my brother continue to be symptom free.

Day 3: I woke up feeling fine. Nasal congestion is moderate in both nostrils. My daughter's voice is more hoarse than yesterday and her nose is producing substantial amounts of mucus. She is coughing and sneezing. My wife and my brother continue to be symptom free.

Sample collection and H₂O₂ inactivation: In the morning I collected more of my daughter's nasal mucus (maybe about 0.25 to 0.3 mL). Using a graduated dropper, I mixed the sample with 2 volumes of hydrogen peroxide (0.5 mL), and incubated for about 30 minutes at room temperature. A couple of times during the incubation period I used the dropper to mix the sample to ensure homogeneity.

Dental floss administration (see [here](#)): Using a floss pick, I flossed between all teeth, rinsed with one mouthful of clean water, and evacuated my mouth of excess fluid by spitting. I used the dropper to deposit about half of the mixture onto my upper gumline. I used a fresh floss pick to work the mixture between my teeth and deeper into the gumline. I repeated the procedure with my lower gumline and teeth. I swallowed the

remaining mixture. I didn't eat or drink anything for 30 minutes. My gums felt a bit sensitive and sore the rest of the day, especially when I clenched my teeth.

A few hours later I applied an intranasal dose of double antibiotic cream

Day 4: I woke up feeling fine but with a slight irritation in my throat. Nasal congestion is moderate to heavy in both nostrils. My daughter's nose continues to produce copious amounts of mucus, but she seems to be coughing and sneezing less. She woke a few times during the previous night. My wife is low energy and her nose is running but otherwise seems ok. My brother says he feels fine.

Sample collection and H₂O₂ inactivation: In the morning I collected more of my daughter's nasal mucus (maybe about 0.5 mL). Using a graduated dropper, I mixed the sample with 1.5 volumes of hydrogen peroxide (0.75 mL), and incubated for about 60 minutes at room temperature (longer than intended but some things got in the way of timely administration). A couple of times during the incubation period I used the dropper to mix the sample to ensure homogeneity.

Dental floss administration: I repeated the dental floss procedure I performed yesterday. I didn't eat or drink anything for 30 minutes. Starting within an hour of flossing, my gums felt a bit more sensitive than usual, especially when I clenched my teeth.

Day 5: I woke up feeling fine but with the slight irritation in my throat has gotten a bit worse. Nasal congestion is moderate in both nostrils. My daughter's nose continues to produce copious amounts of mucus, but she is coughing and sneezing much less. My wife says she feels fine. My brother says he feels fine.

Day 6: I woke up feeling fine and my throat feels better. Nasal congestion is low to moderate in both nostrils. When my daughter speaks she sounds a bit congested, and her nose is still slightly runny, but otherwise she seems fine. My wife says she feels fine. My brother says he feels fine.

Day 7: Everyone in the house seems fully recovered. My daughter sneezed several times in the morning. We'll see later this afternoon if that continues. It might be a new infection.