# Vaccine Refrigeration & Thermal Stability

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## Abstract

Presently, vaccines prevent infection and have eradicated multiple diseases, yet many vaccine-preventable diseases are still a major issue in the developing world.[1] A primary obstacle to making vaccines accessible worldwide is the near-freezing temperatures conventional vaccines must be stored at.[2] Novel technologies are being developed for reliable & efficient refrigeration and to increase vaccines temperature-tolerance. Improved refrigeration, such as off-grid fridges[3,4], can be implemented almost immediately after development, but must keep current vaccines within a narrow temperature range: overheating and freezing can lead to vaccine spoilage[5]. Alternatively, creating temperature-tolerant vaccines via methods like nanotechnology[6] and post-production modification[7] allows higher certainty that the vaccine was not damaged during transport or storage; however each vaccine must be made and approved individually. A combination of the above methods will be the most likely road to success, and give us the best chance of making even more diseases as much of a vaccine success story as smallpox.

## Introduction

When the smallpox vaccine was first developed in 1796, it revolutionized medicine, and current vaccines save up to three million lives annually.[8] However, the World Health Organization (WHO) estimates that 1.5 million children died from vaccine-preventable diseases in 2011 alone, many from low-income countries where vaccines are not readily available.[9] A major issue in making vaccines accessible in these areas is keeping them thermally stable: most vaccines must be kept between 2-8°C,[10] and even a short time outside of this range can deem them unfit for use. Although this is most often not a major issue in developed areas where clinics have easy access to refrigeration and consistent electricity, this constraint can become a major hurdle for less developed areas, as a few hours without power could leave uncertainty about whether their stock of vaccines are still fit for use.[11]

Historically, this has limited the populations which vaccines could cover, but new and innovative technologies on biological and engineering fronts are helping vaccines reach more people and save more lives.[11,12] Solving the issue of keeping vaccines cold has been mainly been approached from two modes of thought: that of improving refrigerators to be cheaper, more long-lasting, and require little or no electricity, and that of improving vaccines to make them stable at and above room temperature for either a short duration or indefinitely. Each method has advantages and disadvantages, with better refrigeration generally best for helping people in the short term and helping people that need vaccines now, and improved vaccines best for proving a more sustainable and reliable solution that will take longer to reach.

**Cold Chain – Current Methods**

Presently, most vaccines must be kept cold (2-8°C) during all stages of transport, storage, and deployment. The 'cold chain' is a system of getting a product (generally food or pharmaceuticals) to the end user, where in each step the temperature must be carefully controlled and monitored. As is shown in Fig. 1, the transport stages of vaccine delivery do not post much of a problem: few vaccines are found to go bad while on route to intermediate destinations. However, 75-83% of all vaccines that go bad are found to spoil in the storage and deployment stages of the cold chain, indicating a great need for reliable large-capacity, long-term storage fridges and improved mobile fridge and coolers. Vaccines which do not need refrigeration would eliminate this need, however, until these are developed, improving fridges to help current populations will greatly help cut down on the amount of vaccines wasted.

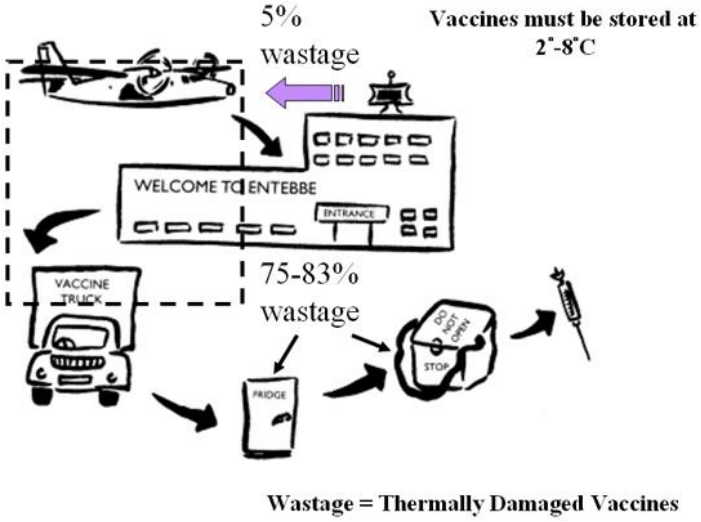


*Fig. 1 - Diagram of the current 'cold chain' vaccine transport system. Percent wastage indicates what percent of vaccine spoiling happens in the corresponding stage. Note that the highest amount of wastage happens in the storage and mobile refrigerator stages of transport, and hence more efforts are focused on those areas. Image from One World Medical Devices.*

**Improving Refrigeration**

Development and use of solar refrigerators

Vaccine refrigerators have come a long way in the past 50 years, and are continuing to progress. Many vaccine refrigerators can now be powered solely using solar energy, [3,4,13] and ones that are still grid or fuel based have specialized batteries to work with inconsistent power. Solar, or photovoltaic, vaccine refrigerators were envisioned by Augustin Mouchot in the mid-19th century,[14] and have been tested and used in the field since the 1970's. At the time, these fridges showed some reliability, but the cost of these refrigerators were very high, they needed a large area for solar panels, and their storage space size was generally small compared to the full volume of the fridge.[15] By 1990, solar power technologies had developed to the point where solar fridges were considered stable and reliable, but no so much as to create a clear divide between solar and a previously used mode of off-grid cooling, kerosene refrigeration. Although kerosene fridges needed a constant fuel supply, created toxic fumes, and didn't meet the WHO Performance, Quality, and Safety guidelines,[16] solar fridges were still very expensive, needed full sun, and required complicated assembly and maintenance.[17] Additionally, the batteries used to store solar energy presented a major issue: their ability to hold charge dropped significantly with time, and the state of charge was very difficult to determine. However, in recent years solar panels, batteries, and insulation have all improved, allowing for more efficient systems that require less space and use less power, making solar refrigeration a feasible and appealing option for many areas with limited access to grid electricity.

Current solar refrigeration

Many developments in the technologies behind solar refrigeration, such as solar panels, capacity, and energy storage options have greatly furthered vaccine refrigeration in the previous decade. [18] As batteries were what went wrong most commonly in previous solar refrigerators,[19] energy storage has been a center of focus in developing more reliable and longer-lasting systems. Adsorption, also known as direct drive, cooling, an alternative to battery-powered refrigerators. Rather than using batteries to store energy, adsorption systems use refrigerants such as ammonia or alcohols to directly generate ice, which is used to cool vaccines. Until recently, adsorption systems were considered technically successful but too expensive to compete with battery-based solar systems,[20] however

recent developments have put increasingly on the market, and made up about half of the solar refrigerators supplied by UNICEF in 2012 (as compared to about 30% in 2010 and less than 5% in 2008).[21] Other developments in solar refrigeration include a solar-gas hybrid system for areas where solar power may be insufficient or inconsistent[22] and advances in passive cooling systems that can be used for approximately without a power source by using nano porous amorphous silica insulation.[23,24]

Two notable solar refrigeration systems are the Sure Chill and Sun Frost refrigerators. The patented Sure Chill system, shown in Fig. 2, uses the property that water is heaviest at 4°C to keep stable refrigeration at that temperature for up to 10 days without power. An ice bank is formed above the refrigerator compartment, and as the ice melts it flows down past the compartment. Additionally, as water heats up past 4°C it rises, meeting the melted water from the ice to keep stably at 4°C. This technology has been applied to multiple facets of both the medical and food industries, including specialized vaccine refrigerators.[13]

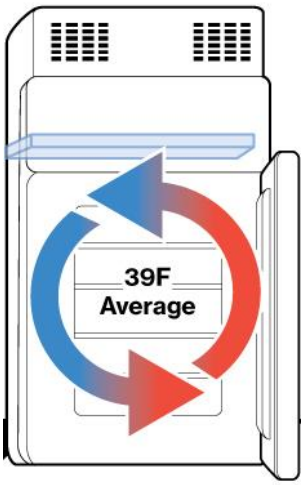


*Fig. 2- Diagram of the Sure Chill refrigeration system. The refrigerator uses an ice bank above and the principle that water is heaviest at 4°C (39°F) to keep a constant supply of water at that temperature flowing around the chamber. This approach can continue refrigeration at a stable temperature for up to 10 days without power. Image from Tozzi.* [25]

The Sun Frost refrigerator is most notable for its advanced efficiency. Due to its low energy compressor and condenser, it takes significantly less power to operate than a standard refrigerator, taking only 0.28 kWh/day at 32°C and 0.43 kWh/day at 110°C [4]compared to household refrigerators which can take from 1.3 to 4.4 kWh/day at room temperature (20°C).[26] The Sun Frost RVFB vaccine refrigerator was also tested by the WHO and PATH for long life batteries, and its current batteries last from 2-15 years, depending on battery quality and usage. Although the system is marketed mainly as solar, any of wind turbines, hydro power, generators, car batteries, or grid electricity can all be used to recharge its batteries. Thus far, Sun Frost refrigerators have been used in over 50 developing nations, since 2002. Although use of these refrigerators (compared to more traditional systems) is difficult to assess, the large body of work being done in this field as well as interest in these programs by organizations like the WHO, PATH, and the Bill & Melinda

Gates Foundation shows a definite interest in improving refrigeration efficieny, especially in regard to solar refrigerators.

Mobile refrigeration

An important phase in vaccine transport, and one in which things often go wrong, is the deployment of vaccines from clinics, to smaller towns and villages. Clinics, and even larger towns, may have large stationary refrigerators, but in reaching populations whose sizes do not justify a full-sized refrigerator, coolers and refrigerators that can be transported by an individual are often the most effective solution. Coolers were previously a standard mode of transport in these cases, however they severely limited the range which smaller amounts of vaccines could be carried due to very small storage space and that they couldn't be cooled again once they became warm.

Multiple mobile fridges are now on the market or in development to fill this gap. Models such as the 25-Liter FFM Medical FridgeFreeze[27] are much like an electric cooler, with similar appearance, and are light enough to be carried by one or two people. A downside is that they often need power semi-regularly, which may not be feasible for longer journeys in certain areas. Others take a similar approach, but design more specifically for longer journeys: the VacPac [28] (Fig. 3) is a backpack-style mobile fridge that holds 1200 doses, can last 18-24 hours from full power, and can be recharged via solar panels, car outlets, or from grid electricity. Bringing mobile cooling even further, Sure Chill was awarded 1.4M USD in 2014 by the Bill & Melinda Gates foundation to design vaccine coolers that can keep vaccines within range for over two weeks without a power supply.[29] The company, known for making various refrigerators used in the food and medical industries, holds patents on an adsorption cooling system that uses the fact


*Fig. 3- The VacPac mobile vaccine refrigerator. Designed to be worn as a backpack, stores 1200 doses of vaccine, and rechargeable batteries allow for 18-24 hours of cooling per charge. Image from One World MD.*

that water is heaviest at 4°C to keep vaccines stably at that temperature, and have potential to apply that technology to vaccine coolers as well.

Refrigerator monitoring

Currently, between 75-100% of vaccines go outside of the cold chain at some point between manufacture and delivery,[30,31] and many of these instances go unchecked. Delivering vaccines that have gone bad cause non-immunogenic vaccinations, resulting in individuals that are believed to be vaccinated but in reality are not. Accurate monitoring of entire refrigerators can significantly decrease the amount of spoiled vaccines that get distributed.

Whole fridge monitoring helps ensure that batches of vaccines are kept at appropriate temperatures throughout the cold chain. Some vaccine fridges now come with thermostats to help ensure that temperatures fluctuations are caught, and it is recommended by the CDC to check fridge and freezer thermostat values twice daily.[32] Although this helps prevent and discover large fluctuations, there are still issues with many vaccines being frozen or overheating. A solution currently employed by the WHO is the Thermco Fridge-tag temperature monitor,[33] shown in Fig. 5, specifically designed to help clinicians store and record vaccine fridge data. This device records stores temperature data for 30 days and allows clinicians to upload data to a computer to check for any possible missed cold chain breaches. Although this solves the problem of missed temperature lapses, and includes alarms to alert nearby clinicians when the temperature becomes unsafe, it still cannot save vaccines if temperature changes overnight.


*Fig. 4 – The Fridge-tag 2 temperature monitor. The device records temperature for 30 days from a digital thermometer placed in a dummy vaccine vial. The USB shown behind allows clinicians to upload data to a computer to check for any temperatures breaches that may have been missed. Image from Thermco Products.*

A device being tested to combat this issue is a ColdTrace vaccine monitor,[34] which is stored with vaccines, allows monitoring at all stages, and additionally sends SMS text messages to those responsible for care of the vaccines when the cold chain is breached for a specific fridge or batch. This technology is currently under trial in over 150 sites in Kenya, Mozambique, India, and the Asian Pacific, with 350 additional deployments planned for 2015. Significant breaches in several clinics in Kenya have already been discovered, as shown by the large spikes out of the dashed-line acceptable regions in many clinics seen in Fig. 5, and improved refrigerator monitoring should help clinics identify weak points in their refrigeration systems and lose less vaccines.

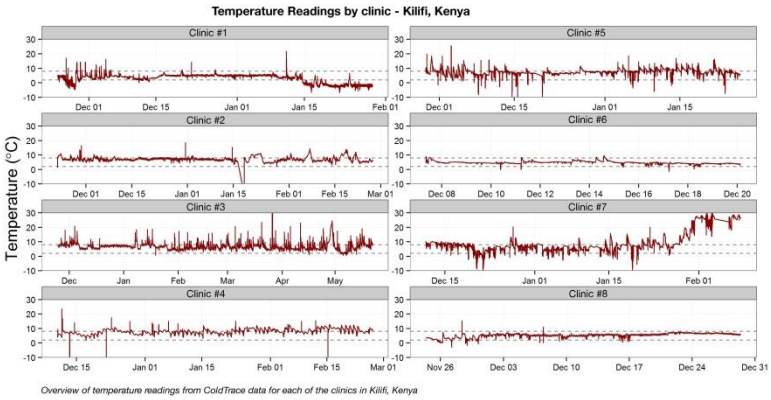


*Fig. 5 – Temperature recorded by a ColdTrace vaccine fridge monitor from eight clinics in Kilifi, Kenya. Many clinics show large deviations from the accepted cold chain range (shown by gray dashes) as well as cyclic spikes out of range, indicating severe weak points in refrigeration systems. Image from Datakind.*

Although this would greatly benefit clinicians and those receiving vaccines alike, it still leaves room for improvement. An important next step could be integrating these monitoring systems with a control method for the refrigerators themselves. Although some incidences of temperature breach are caused by accidents or external factors, cases related to natural fridge processes could be greatly reduced or eliminated by directly incorporating systems like the Fridge-tag and ColdTrace monitors into the cooling system controls of today's refrigerators.

## Individual vial monitoring

As much as whole-fridge monitoring can prevent large-scale vaccine loss, our current system is far from disaster proof. When a fridge or cooler was found to be compromised, entire batches of vaccines would have to be thrown out, and testing vaccines by sending samples to labs for immunogenicity (usually done in mice) is often comparable to or more expensive than a batch of new vaccines. This is where individual vial

monitoring comes in: being able to tell specifically which vaccines were damaged helps conserve vaccines and save as many lives as possible. Additionally, it can notify clinicians and distributors if individual vaccines have gone bad even if no noticeable breach has occurred, preventing harmful use of spoiled vaccines.

The greatest success story in this space so far has been the PATH-based Vaccine Vial Monitor, which saves the global health market an estimated $14 million per year.[37] This temperature-activated sticker is placed on vials of most modern vaccines, and, as displayed in Fig. 6, the color of the center of the sticker gets darker as it's exposed to temperatures outside of the cold chain, where higher temperatures cause faster color changes. When the center square becomes or passes the color of the surrounding circle, as shown in the third frame, the vaccine is no longer effective, and the monitors are manufactured specifically to each type of vaccine labeled.[38] It is estimated that this simple technology will reveal over 200 million damaged vaccines in the next decade, ensuring as many children are properly vaccinated, and has already saved millions of effective vaccines from being thrown away, including 50,000 in a single instance in Indonesia.[37]

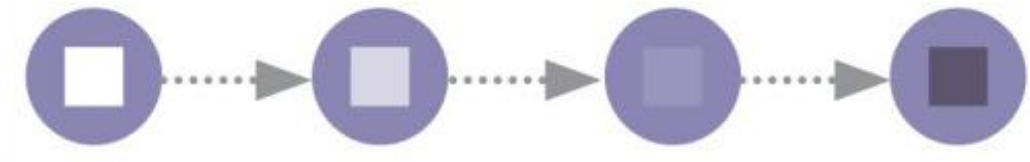

*Fig. 4 – The Vaccine Vial Monitor, a sticker developed by PATH to help clinicians determine if a vaccine is fit for use. The center square gets darker as the vaccine is exposed for longer times or to more intense temperatures outside the cold chain, and the vaccine is determined unfit for use when the color of the square matches the color of the circle. Image from PATH (36).*

**Improving Vaccines**

Although better refrigeration systems greatly help in getting vaccines to many places, they are many vaccination challenges they cannot solve. They are far from foolproof, and many accidental temperature changes still occur even with precise monitoring and care. A door left open or a long-term power failure could destroy batches at a time, and as previously noted, over 200 million vaccines will be found to be damaged in the next ten years.[37] Additionally, cold-chain dependent vaccines require clinics to purchase specialized refrigerators and find ways to power these fridges reliably, a major challenge in some areas. Refrigeration systems must also be developed and paid for during

all transport stages of vaccine delivery, both before and after the clinic. However, if vaccines were not cold chain dependent, many of these accidents would not be an issue, and refrigeration costs could be circumvented. MenAfriVac has been the most notable success of this approach yet, and nanovaccines as well as other alternative stabilization methods being heavily researched. Once developed, temperature-stable vaccines can provide a more reliable and efficient long-term solution.

MenAfriVac

Meningitis, a disease caused by the microbial infection of brain and spinal cord membranes, causes neck pain, fever, vomiting, and other general illness symptoms. It is most common in sub-Saharan Africa , and young children have the highest risk of acquiring the disease. The disease can be fatal for 1 in 10 cases, and during epidemics up to 1% of the population can become infected. In Africa's "meningitis belt," 25,000 people died of meningitis in 1996-7 alone. Over 90% of meningitis epidemics that occur in this area is caused by the *Meningococci* serotype A, an agent that no longer presents much of an issue for most well-immunized areas, however, the hot temperatures and lack of stable electricity for many areas in this part of Africa has made vaccination very difficult.[39,40]

The previous polysaccharide (PS) vaccines for meningitis only gave short term immunity, didn't prevent people from becoming contagious carriers of the disease, and were non-immunogenic in infants. The Serum Institute's conjugate vaccine solved these issues, and was found to be more immunogenic than the previous vaccines available, and no cases of group A meningitis were found in the vaccinated population in 2011. Conjugate vaccines are typically more immunogenic than PS vaccines, especially in infants, due to chemical modification of the viral capsule PS and addition of a protein which serves to attract and stimulate CD4 helper T cells.[41] Additionally, a major contributing factor was that it can leave the cold chain and be exposed to temperatures up to 40°C for up to four days and still be effective.[42,43] This has greatly helped to prevent vaccine loss due to storage accidents, and deliver vaccines to many places previously considered inaccessible. Although many facets of this vaccine make it nearly ideal for its target community, four days of temperature tolerance still requires a heavily monitored controlled temperature

chain, and current work is being done to make vaccines that will be stable for longer periods of time, possibly as long as the vaccine lasts.

In 2010, the Serum Institute of India, along with the organization PATH, began releasing the MenAfriVac vaccine, the first vaccine specifically designed for thermal tolerance in Africa.[44] In a massive vaccination effort since then, over 150 million people in the meningitis belt have been vaccinated.

Nanovaccines

One promising new avenue and large area of research is the application of nanotechnology to vaccines, commonly termed 'nanovaccines.' Nanovaccines vary in type of antigenic function, with nano-beads and DNA vaccines at the forefront of the field. Nano-beads are inert nano-scale particles with an antigen conjugated to the surface, and have been demonstrated to initiate humoral and cell-mediated immunity, including stimulation of CD8 T cells.[45] A nano-bead vaccine developed in 2011[6] showed considerable efficacy in mice, and are stable at room temperature for six to ten months[46], leaps and bounds better than current thermally stable vaccines. Nanoparticle vaccines achieve stability through choice of suitable polymers, which are used to allow controlled release of the encapsulated antigens and adjuvants, and have the useful side effect of keeping the dry form of encapsulated vaccines stable for at least four months anywhere between -20 and +40°C, resulting in vaccines that can be delivered along with cold chain vaccines or at ambient temperatures without risk.[47] Nanovaccines also allows for a single-dose intranasal platform, as due to their small size they can be absorbed through mucosal tissue. This would not only induce mucosal as well as systemic immunity, but also greatly booster patient compliance and make it much more suitable for developing areas by removing both biohazard waste and the need for needle-trained health professionals to administer the vaccines. Nanoparticles are also being tested for the preemptive development of a vaccine for H5N1 avian influenza, which is not yet able to infect humans, but could be very dangerous should it do so.[48] Additionally, antigen-presenting microspheres are under development as a delivery method, which have similar properties to nanoparticles and have shown to be immunogenic in mice.[49]

DNA vaccines are plasmids injected intramuscularly that cause the body to create a specific antigen for a short time, often yield long-lasting immunity. They also make excellent candidates due to low manufacturing costs, and Phase 1 & 2 clinical trials have been completed for HIV, HBV, HVC, HSV, tuberculosis, and malaria, as well as various cancers.[50] Microneedle arrays are another method being examined for delivery of DNA vaccines, where the arrays are coated in the plasmid for subcutaneous delivery.[45]

Alternative stabilization methods

Another promising method that has shown stability at 45°C for up to six months is resuspending vaccine particles in carbohydrate glass. The particles are taken from liquid solution and dried onto a membrane of glass fibers, which can then be stored until ready for reconstitution in a saline solution and use. This allows for shipping and storage of the vaccine as a syringe of buffer which can attach to a glass filter membrane, both of which can withstand ambient temperatures for extended periods of time, potentially between 7 and 12 months. Immunogenicity has been shown of live poxviral and adenoviral vaccines in mice through this method, and if it is safe and effective in humans, would be a great asset in making applicable current vaccines thermally stable. [7]

Reevaluating current vaccines

Although many vaccines have been tested for temperature tolerance and found to be cold-chain dependent, it appears that not all vaccines previously though to need a strict cold chain are actually as dependent as previously thought. A study conducted in 2012-2013 by Epicentre, the research branch of Medecins sans Frontiéres (MSF, also known as Doctors Without Borders),[51] showed that the tetanus toxoid vaccine was stable for up to 40°C for up to 30 days via tetanus antibody measures.[52] Multiple other vaccines are being tested similarly, in hopes that many of them will also be more thermally stable than previously believed, reducing our reliance on the cold chain. This and similar discoveries can provide a faster and more efficient way of improving vaccine delivery, as completing this process for multiple vaccines can give refrigerators and operators more leeway without the need for discovery and full clinical testing of a 'new' drug.

## Design Principles

The ideal solution to issues with vaccine refrigeration and thermal spoilage would be vaccines whose stability is not temperature dependent at all. If the cold chain was to become completely unnecessary, this would not only lift a huge financial burden from developing countries and organizations like MSF and PATH, but also allow greater access to vaccines and assurance of their quality. However, the very nature of vaccines as biological materials creates hurdles for this path, and greatly increases both the time and effort that it will take to create this solution. This is where better refrigeration comes in, as a way to focus on the people now whose lives will be saved and improved through vaccines, and help spread current vaccines as widely as possible.

## Conclusions

Improving vaccine refrigeration and decreasing need for a strict cold chain will save millions of lives and prevent many diseases, potentially even leading to eradication of current vaccine preventable diseases that still run rampant in parts of the developing world. Moving forward, it will be important to continue to improve refrigeration technologies, especially in the short term, to allow further coverage and less wastage of current cold chain dependent vaccines. However, in the long term, vaccines whose shelf lives are not temperature dependent would be an ideal solution, as this would remove need for a cold chain at all, and with it remove the costs and uncertainty associated with current refrigeration. Additionally, this allows us to develop vaccines which can be administered orally or intranasally, which would greatly help vaccine delivery systems. We are currently very far from this goal, but improvements in vaccines push us closer to vaccines that have no risk of spoilage due to temperature.

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