

# Opportunities and challenges of developing thermostable vaccines

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All vaccines lose potency over time and the rate of potency loss is temperature-dependent. Therefore, cold-chain systems have been established to ensure that the potency of vaccines is maintained by storing them under refrigerated conditions (in most cases between 2 and 8°C) until the point of use. This article aims to review the approaches being used to develop thermostable vaccine formulations that would be resistant to damage caused by freezing or excessive heat, and that could reduce dependence on the cold chain. The challenges associated with the implementation of these novel formulations are discussed, as well as the potential benefits and opportunities of taking vaccines out of the cold chain.

**KEYWORDS:** adjuvant • cold chain • freeze-exposure stability • heat damage • reformulation • spray-drying • thermostable • vaccine

## Stability of commonly used vaccines

For the majority of existing, commonly used vaccines, a shelf life of 2 years or longer at 2–8°C is possible. However, the sensitivity of vaccines to excursions outside of this range varies widely (TABLE 1). Furthermore, lyophilized vaccines are generally only stable prior to reconstitution. Once they have been reconstituted, the potency of live-attenuated vaccines such as those for measles and yellow fever can drop rapidly. WHO recommendations state that reconstituted vaccines must be kept cold and any unused vaccine from a multi-dose vial must be discarded within 6 h [101]. This policy is related in part to the instability of reconstituted vaccines, but also minimizes the chance of bacterial contamination because live vaccines do not contain preservatives.

## Temperature excursions in the cold chain

Experience has shown that it is possible to establish a cold chain for vaccine storage and distribution in any country. However, the effort is a major undertaking, maintenance is difficult and the capacity of the existing systems is under increasing pressure as new vaccines are developed and introduced into lower- and middle-income countries.

It is also apparent that, despite best efforts, cold chains do not always function as intended for many reasons, such as improperly maintained or outdated refrigeration equipment, loss of power

or lack of fuel to operate equipment, poor compliance with cold-chain procedures, inadequate monitoring and poor understanding of the dangers of vaccine freezing [1]. The result is that vaccines in the cold chain are sometimes accidentally subjected to temperatures both higher and lower than the target range.

## Freeze exposure of vaccines

Cold-chain operations have historically focused on protecting vaccines from excessive heat, with the result that inadvertent freezing is now considered to be the most important problem affecting vaccine integrity [1]. This is an issue of growing importance as the number of expensive freeze-sensitive vaccines used in immunization programs increases; it is estimated that freeze-sensitive vaccines represented over 31% of the US\$439 million that the UN Children's Fund spent on all vaccines in 2005 [2].

A recent systematic review of published cold-chain studies found that freezing temperatures were encountered during transport (35.3%) or storage (21.9%) of all vaccine shipments monitored in developing countries [2]. Studies that analyzed cold-chain conditions from national or regional stores all the way to health clinics found that 75–100% of the shipments were exposed to freezing temperatures at least once during the distribution process [2]. Monitoring of the cold chain

**Table 1. Stability of commonly used vaccines.**

Vaccine	Formulation	Freeze sensitive	Stability at:			
			2–8°C	25°C	37°C	≥45°C
Diphtheria and tetanus toxoids	Liquid, aluminum adjuvant, usually in combination vaccines	Yes	Stable for >3 years	Stable for months	Stable for months	Unstable above 55°C
Hepatitis B	Liquid, aluminum adjuvant	Yes	Stable for >4 years	Stable for months	Stable for weeks	Stable for days at 45°C
Pertussis (whole cell)	Liquid, aluminum adjuvant, always in combination vaccines	Yes	Stable for 18–24 months	Stable for 2 weeks	Stable for 1 week	≥10% loss of potency per day
Meningococcal serogroups ACWY (polysaccharide)	Lyophilized, no adjuvant	Yes	Stable for 2 years	Stable for weeks to months	Not known	Not known
<i>Haemophilus influenzae</i> b, conjugate	Liquid or lyophilized, no adjuvant or in combination vaccines	Yes (liquid)	Stable for >2 years	Stable for >2 years (lyophilized)	May be unstable, depends on presentation	Unstable
Pneumococcal, 7-valent, conjugate	Liquid, aluminum adjuvant	Yes	Stable for >2 years	No data	May be unstable, depends on presentation	Unstable
BCG ( <i>Mycobacterium tuberculosis</i> )	Live-attenuated, lyophilized, no adjuvant	No	Stable for 1–2 years	Stable for months	Loss of >20% potency after 1 month	Unstable
Measles, mumps and rubella	Live-attenuated, lyophilized, no adjuvant	No	Stable for 2 years	Stable for >1 month	Stable for >1 week	Unstable
Yellow fever	Live-attenuated, lyophilized, no adjuvant	No	Stable for >2 years	Stable for months	Stable for >2 weeks	Unstable
Oral poliovirus	Live-attenuated, liquid, no adjuvant	No	Stable for ≤1 year (Stable for ≥2 years at -20°C)	Stable for weeks	Stable for 2 days	Unstable
Inactivated poliovirus	Liquid, no adjuvant, can be in combination formulations	Yes	Stable for 1–4 years	Stable for weeks	Stable for weeks	Little data available
Rotavirus	Live-attenuated, lyophilized or liquid, no adjuvant	No	Stable for >2 years	Stable for 2 years	Not known	Not known
Influenza, inactivated	Liquid, no adjuvant	Possibly	Stable for up to 1 year	Not known	Not known	Not known
Japanese encephalitis B, live	Live-attenuated, lyophilized, no adjuvant	No	Stable for 1.5 years	Stable for 4 months	Stable for 7–10 days	Unstable
Human papillomavirus virus [33]	Liquid, aluminium adjuvant	Yes	Stable for >3 years	Stable for >2 years	Stable for 1.5 months	Stable for 14 days

All data from [101] unless stated otherwise.  
All vaccines are nonlive unless stated otherwise.  
BCG: Bacille Calmette–Guérin.

in Papua New Guinea found similar results: 100% of all vaccine shipment studies were exposed to freezing temperatures at some point, although exposure to excessive heat was rare [1].

Accidental freezing can occur when vaccines are placed too closely to the walls of ice-lined refrigerators, placed too closely to the evaporator in other types of refrigerators or placed with

frozen ice packs inside insulated containers for transport. If ice packs are not preconditioned (i.e., allowed to start to melt) prior to packing, then vaccines placed close to the packs without sufficient insulation are exposed to temperatures below the freezing point of the vaccine. It is a common belief that inadvertent freezing generally goes undetected, despite the availability of tools such as the shake test, a simple procedure based on using sedimentation rates to detect whether adsorbed vaccines have been frozen and therefore damaged [102].

### Impact of exposure to freezing temperatures

Evidence from *in vitro* and *in vivo* laboratory studies has demonstrated that freezing of aluminum salt-adjuvanted vaccines damages the vaccine–adjuvant matrix; formation of ice crystals overcomes repulsion forces between aluminum particles, resulting in coagulation and agglomeration of particles and an overall increase in particle size [101]. The net result is a reduction in the potency of the vaccine [2,3]. Conclusive data showing that vaccine failure in the field has occurred as a result of freezing has, however, been more difficult to obtain. Indirect evidence has been obtained from studies of vaccinations against hepatitis B in Mongolia. Hepatitis B vaccine transported to rural settings in Mongolia was found to be exposed to freezing temperatures more frequently than vaccine used in urban settings [4], possibly explaining the difference in proportions of children with an adequate antibody response to the hepatitis B vaccine from rural (71.4%) and urban (94.6%) settings [5].

Exposure of vaccines to subzero temperatures, however, does not necessarily equate to vaccine damage. Recent work has shown that freeze-induced damage to vaccines is a complex process, dependent on many factors [3]. The studies used a commercially available hepatitis B vaccine with a freezing point of  $-2.8^{\circ}\text{C}$ . After storage at  $-6^{\circ}\text{C}$  for 72 h, the vaccine did not freeze, providing it was left undisturbed. Agitation of the vaccine (as would be expected during transportation) caused the vaccine to freeze completely within 3 h at  $-6^{\circ}\text{C}$ . Furthermore, repeated freeze–thaw cycles were needed before the potency of the vaccine was affected, as assessed by *in vitro* assays or *in vivo* immunogenicity: three 20-h cycles at  $-10$  or  $-20^{\circ}\text{C}$  followed by thawing led to 2.6- and 7.2-fold reductions in antibody titer, respectively [3]. Loss of potency correlated with damage to the aluminum adjuvant, as indicated by particle aggregation measured by the sedimentation assay and particle size analysis. Although some storage conditions (e.g.,  $-2^{\circ}\text{C}$ ) did not induce measurable acute damage to the vaccine, it is possible that these conditions may still have had an impact on the long-term stability of the vaccine.

### Heat exposure of vaccines

Even though aluminum salt adjuvants are thought to be unaffected by high temperature, excessive heat can damage vaccines in a number of ways, altering the tertiary structure of proteins, causing dissociation of polysaccharides from the protein carrier in polysaccharide conjugate vaccines and, in the case of live-attenuated vaccines, reducing infectivity.

The heat damage to a vaccine could be the direct result of inadvertent exposure to elevated temperatures or, in the case of lyophilized vaccines, heat shock from the addition of diluent that is too warm. The sensitivity of different vaccines to damage by heat varies widely: human papillomavirus (HPV), diphtheria, tetanus toxoid and hepatitis B vaccines are the most heat-stable; freeze-dried measles, yellow fever and bacille Calmette–Guérin (BCG) vaccines have moderate stability; oral poliomyelitis vaccine is the most heat-labile (TABLE 1). In all cases, the impact of heat damage is cumulative: the higher the temperature and longer the duration of exposure, the more extensive the degradation. Vaccine vial monitors (VVMs) measure cumulative heat exposure and indicate whether a vaccine has been exposed to excessive temperature over time and whether it is likely to have been damaged as a consequence. VVMs with reaction rates specific to four different models corresponding to four groups of vaccines based on their heat stability have been developed. These were introduced in the late 1990s and are now used by most vaccine manufacturers [101]. Training of healthcare workers in the use and interpretation of VVMs has also allowed certain vaccines to be used outside the cold chain (OCC; discussed in more detail later).

### Stabilization methods

Vaccine formulations that are resistant to heat damage would have major benefits, including reducing vaccine wastage, helping to ensure the effectiveness of vaccines, being less dependent on cold-chain supplies and equipment, extending the outreach of immunization programs by facilitating OCC delivery of some vaccines and enabling vaccination activities to continue in emergency situations (e.g., earthquakes or tsunamis) when the cold chain might well break down [6].

### Developing thermostable liquid formulations

Liquid formulations of vaccines have been the ‘default’ presentation since the introduction of vaccines. The first vaccines to be used were liquids and, as more vaccines became available during the 20th Century, production of liquid formulations continued, which was the most straightforward approach, avoiding the need for new formulation development and manufacturing equipment. Freeze-dried formulations were only produced if necessary to achieve adequate stability. Today, liquid vaccine formulations are still preferred over dry formulations owing to the relative ease of their manufacture, packaging and use. Therefore, thermostable liquid formulations have the significant advantage of being compatible with existing vaccine manufacturing processes.

Most of the existing liquid vaccine formulations have been developed for storage under refrigeration, but not at higher temperatures, with the result that their stability may not be optimal. For example, most current liquid vaccine formulations do not use any stabilizers in spite of their beneficial effects on thermostability, as seen in research studies.

New methods are now being advanced that can be used to develop liquid vaccine formulations with high precision and maximal possible stability. Using high-throughput screening methods,

the stability of vaccines in liquid formulations can be maximized by optimizing the properties of the solvent (e.g., buffer, pH and salt concentration) and by the addition of stabilizing excipients. These excipients can stabilize proteins by a number of mechanisms, including buffering against pH changes, preferential hydration (e.g., nonreducing sugars such as sucrose and trehalose), decreasing adsorption and aggregation (e.g., nonionic surfactants) and by providing steric hindrance of protein–protein interactions (e.g., polymers and protein stabilizers such as serum albumin).

### **Causes of vaccine instability in liquid formulations**

Instability of proteins (and therefore of protein vaccines) in solution is due to a number of physical and chemical processes. The most significant is probably unfolding, leading to alteration of quaternary, tertiary and secondary structure, and subsequent aggregation of partially denatured proteins, which will occur to minimize unfavorable thermodynamic interactions. Chemical instability owing to unwanted reactions such as hydrolysis, oxidation, deamidation and the breakage or formation of disulfide bonds can also result in loss of vaccine potency. All of these destabilizing processes are influenced by factors such as pH, buffer, salts and ionic strength, and are accelerated by temperature increases [7].

Live-attenuated vaccines in liquid formulations are extremely unstable owing to the need to maintain viability of the infectious organism. They are susceptible to inactivation by the same destabilizing processes as before, which can disrupt the conformation and function of structural proteins and glycoproteins, adversely affecting viability and infectivity. In addition, the lipid bilayer envelope of viruses such as the measles virus is extremely susceptible to damage, with the result that enveloped viruses are generally regarded as more labile than nonenveloped [8]. For this reason, the majority of live vaccines are freeze-dried.

### **High-throughput screening tools for developing liquid formulations**

Selection of formulation conditions such as pH, buffer, salt, concentration and stabilizers to provide heat stability or freeze protection to vaccines has been largely empirical and somewhat inefficient to date. The reason is that traditional vaccine potency assays, which often involve immunogenicity and challenges in animal models, are costly and time-consuming. High-throughput formulation screening methods based on protein-structural assays are being developed and these methods offer the advantage of rapid formulation development with great precision.

Relatively efficient methods for comparing different formulations that could speed up the screening process have been proposed, including comparisons of protein-unfolding temperature, half-denaturation concentration and infrared spectra [7]. However, the results from these methods should be treated with caution; protein stabilization has to be approached on a case-by-case basis and screening assays must be selected that correlate with the immunogenicity of the vaccine.

Peek *et al.* have described a system whereby a number of physico–chemical assays – including high-resolution second-derivative absorbance spectroscopy, circular dichroism and fluorescence

spectroscopy – are used to monitor structural changes of protein vaccines undergoing various stresses of temperature, buffer, salts and pH [9]. On the basis of these results, the stability profile of the protein is created to aid the selection of preformulation conditions favoring the stability of the vaccines. Furthermore, these high-throughput structural assays can be used to screen excipients with a prior history of safe use in approved drug products (i.e., generally regarded as safe [GRAS]) or a combination of excipients that further improve the stability of the vaccines under temperature stress. Using this approach, Peek *et al.* were able to screen 32 GRAS compounds, singly and in combinations, for their ability to stabilize the candidate malaria vaccine EBA-175 RII-NG against aggregation at 45°C [9]. The approach has also been applied to respiratory syncytial virus particles [10], measles vaccine [11] and *Clostridium difficile* toxoids [12]. This systematic approach can be undertaken in 1–2 months, enabling the selection of suitable excipients for the formulation of vaccine candidates at an early stage of development. However, while the physico–chemical assays might provide some indications of the vaccine stability, their relevance to vaccine potency *in vitro* or *in vivo* needs to be established on a case-by-case basis.

Another high-throughput screening method has been described to identify stabilizing conditions for liquid formulations of measles virus vaccine. This method, originally designed to optimize drug formulations [13], uses automated high-throughput in combination with a viral infectivity assay as an end point. The strength of this system is that the specific potency assay for the vaccine is employed and the resulting formulation is more likely relevant. The results obtained using this method have not yet been published. Clearly, the challenge of adopting such a system is dependent on the availability of a suitable high-throughput potency assay for each vaccine.

### **Stabilization by minimizing the impact of proton-exchange reactions in aqueous formulations**

One mechanism proposed for the stabilization of proteins in aqueous environments is based on mitigation of the detrimental effect of the continuous proton exchange that occurs between the protein and the aqueous environment. Each proton exchange at the protein surface leads to a temporary change of charge that, over time, encourages the protein to adopt unusually charged states from which denaturation and aggregation are likely to occur. This effect can be controlled by specific buffering systems, leading to enhanced stability of the protein. This concept has been demonstrated using the relatively heat-stable hepatitis B vaccine formulated with aluminium hydroxide. By solely using excipients that have a prior history of safe use in approved drug products (i.e., GRAS), Jezek *et al.* developed a formulation of hepatitis B vaccine containing histidine 40 mM and phosphate 40 mM with a pH of 5.2 (JEZEK *ET AL.*, UNPUBLISHED DATA). The vaccine was shown to be stable for 9 weeks at 55°C and for more than 6 months at 37 and 45°C, as assessed by the standard *in vitro* assay for the integrity of the hepatitis B vaccine. The applicability of the same technology to other vaccines, including *Hemophilus influenzae* type b (Hib) and the combination diphtheria–tetanus–pertussis (DTP)–hepatitis B–Hib, is now being evaluated.

### Freeze-protected liquid formulations for vaccines containing an aluminum adjuvant

In liquid vaccines formulated with an aluminum adjuvant, water molecules form a hydration shell surrounding the antigen and adjuvant particles, helping to preserve the antigen in a native form and the adjuvant particles as a colloidal suspension. Ice crystal formation during freezing disrupts the hydration shell by depleting water molecules, and results in protein unfolding and aggregation of adjuvant particles. Prevention of this aggregation, for example, by the addition of stabilizers that could replace the hydration shells, might result in the maintenance of vaccine potency. Recent studies have identified low-cost and safe excipients that are effective at preventing freezing or preventing freeze-induced damage to vaccines containing aluminium adjuvants [14].

In these studies, propylene glycol (PG), polyethylene glycol (PEG)300 and glycerol were each shown to preserve the structural integrity of the aluminium hydroxide adjuvant in the hepatitis B vaccine during three freeze–thaw cycles (–20 to 25°C). PG was tested for its ability to stabilize hepatitis B vaccine during freeze–thaw cycles to temperatures as low as –10°C (believed to be the lowest temperature to which this vaccine has been inadvertently exposed while in the cold chain). A PG concentration of 20% was found to prevent freezing without any adverse effect on the activity of the vaccine. Formulations containing lower concentrations of PG (2.5–10%) did undergo physical freezing, but retained full potency and had no particle aggregation [14].

Preliminary experiments conducted with two different diphtheria, tetanus and acellular pertussis (DTaP) vaccines, formulated with either aluminium hydroxide or aluminium phosphate adjuvant, indicated that PG, PEG300 or glycerol were each able to prevent damage to DTaP (as indicated by particle-size analysis), suggesting that this approach could be widely applicable to vaccines containing aluminum salts, including pneumococcal conjugate, human papillomavirus and meningococcal vaccines, although the compatibility of the stabilizers with the antigen must be carefully evaluated on a case-by-case basis.

### Developing thermostable dry formulations

Some vaccines, notably many live-attenuated vaccines, are particularly unstable unless stored as a dry product with a low residual moisture content (typically <3%) [8]. Lyophilization or freeze–drying is the most commonly used method for drying vaccines and other biopharmaceuticals. Typically, excipients such as sucrose, amino acids or proteins such as gelatin or serum albumin are used to protect the vaccine during the drying process and storage. Lyophilized vaccines are freeze-resistant

and reasonably stable if stored in the cold chain. Once the dried product has been reconstituted, potency often decreases rapidly, especially if the product is not kept cold [10].

A number of alternative drying processes such as spray–drying (SD), spray–freeze drying (SFD), vacuum–foam drying (VFD) and supercritical fluid drying (SCFD) are being evaluated for their suitability for the production of dry vaccine formulations with the goal of enhancing the thermal stability (TABLE 2) and/or enabling novel vaccination methods such as inhalation. Although the drying methods listed in TABLE 2 involve a number of different processes, each aims to produce vaccine formulations with unique characteristics by removing water molecules from the vaccine suspension while minimizing stresses imposed by excessively high or low temperatures.

A range of excipients has been employed in the stabilizing formulations for dried vaccines, but some general features apply to many of the approaches. Nonreducing sugars, such as trehalose or sucrose, with relatively high glass-transition temperatures are typically used as the primary excipient. Upon drying, these sugars form glass as opposed to crystals to maximize the stabilization effect. Trehalose and sucrose have been shown to be very effective stabilizers and are believed to act by hydrogen bonding to the dried protein and acting as a water substitute [15]. It has been proposed that the amorphous character of the glass enables the intimate contact required for the formation of hydrogen bonds to occur between the sugar and protein [16,17]. Alternative hypotheses, including vitrification [18] and particle isolation [19], have also been proposed to explain how glass-forming stabilizers preserve proteins. Polymers such as PEG, dextran and polyvinylpyrrolidone can aid the stabilization process, either by raising the glass-transition temperature, inhibiting crystallization and/or preventing protein aggregation [20].

Considerable success has been obtained using stabilizing inactivated subunit protein and protein–polysaccharide conjugate vaccines. However, developing thermostable dry formulations of live-attenuated vaccines has proved to be more problematic, presumably owing to their more complex structures and the requirement of maintaining their viability.

Spray–drying has been used to produce a powdered formulation of BCG with improved stability compared with the standard lyophilized formulation. In this case, leucine was used as the sole

**Table 2. Drying processes used to produce thermostable vaccines.**

Process	Vaccines investigated	Stability results	Ref.
Spray–drying	HepB	>24 months, 37°C	(CHEN D, UNPUBLISHED DATA)
	Meningococcal A	>16 weeks, 40°C	(CHEN D, UNPUBLISHED DATA)
	Measles	2 weeks, 37°C	(CHEN D, UNPUBLISHED DATA)
	BCG	4 months, 25°C	[21]
Spray–freeze drying	Split influenza	12 weeks, 40°C	[37]
	Split influenza	26 weeks, ambient	[38]
Vacuum–foam drying	Live LaSota virus	21 days, 37°C	[20]
SCF drying	Measles	1 week, 37°C	[39]
BCG: Bacille Calmette–Guérin; HepB: Hepatitis B; SCF: Supercritical fluid.			



excipient, possibly reducing osmotic damage to the organisms during the SD process [21]. However, it is very likely that the composition of stabilizing formulations and the drying parameters used will need to be customized for each vaccine. For optimal results, live viruses or bacteria might require unique stabilizers that are able to protect membranes and internal molecules such as nucleic acids, as well as external structural proteins. Techniques such as ultrasound [22,23] and electroporation [24] might also be necessary in order to introduce stabilizers into viruses and bacteria so that they can stabilize internal components. At this point, it still remains to be seen whether these approaches will eventually allow the development of thermostable formulations of live-attenuated vaccines.

### **Considerations for selecting liquid or dry formulations**

All dry vaccine formulations currently in use require reconstitution with a liquid diluent prior to administration. While reconstitution might appear to be a straightforward process, in practice it is known to be a potential source of errors: the correct diluent is not always matched to the vaccine, incorrect volumes of diluent might be used, reconstitution might not be undertaken sterilely or multidose vials can become contaminated as doses are withdrawn from the vial. Other undesirable cost and logistical considerations for reconstituted vaccines include the need to use and dispose of reconstitution syringes and needles, and the fact that the packaging volumes are greater than for liquid vaccines owing to the need to have two containers. Work is underway to develop and evaluate simple-to-use reconstitution devices that would reduce or eliminate errors. These devices could have a significant impact on the potential acceptability and uptake of dry thermostable vaccines. In the short term at least, stable liquid vaccine formulations for both oral and injectable delivery have a definite advantage over dry formulations in terms of ease of use, packaging volumes and safety, and are always the first choice.

Drying processes such as SD, SFD and SCFD allow the particle size in the final powder to be controlled. Dry powder formulations, particularly those with a defined particle size, are not only more stable than the liquid formulations, but also are potentially compatible with a range of novel routes for vaccine delivery, such as aerosols, dry powder jet injection, coated microneedles and biodegradable implants. There is a desire to move toward the use of new delivery technologies such as these to eliminate the risks and consequences of accidental needlestick injuries during the processes of injection and waste management, and owing to the deliberate misuse of needles [103]. Thus, in the longer term, development of thermostable powders is a critical step in this process. Unlike lyophilized or vacuum-dried products, which are dried in their final quantities in their final containers, powders produced by SD, SFD and SCFD can be dispensed in varying quantities, thereby offering flexibility in packaging and delivery.

### **Considerations for developing thermostable vaccines**

The public-health benefits of thermostable vaccines might be indisputable, but these do not necessarily translate into commercial benefits for vaccine manufacturers. The technical,

manufacturing and regulatory challenges associated with the development of thermostable formulations of existing vaccines cannot be underestimated (TABLE 3). In cases where there is an existing, effective, low-cost vaccine in routine use, it is likely to be commercially difficult to justify the time and expense involved for development, clinical testing and obtaining regulatory approval for a new thermostable formulation. Changing the formulation of a vaccine with an established safety record may change the safety profile of the product. Thus, manufacturers are likely to be reluctant to change the formulation or manufacturing process for an established low-profit-margin vaccine.

Therefore, it is important that thermostability is given priority early in the development of new vaccines to increase the chance that stability improvements can be incorporated into the final products. Employing high-throughput formulation and freeze-protection methods (in the case of vaccines containing an aluminum adjuvant) should facilitate the development of thermostable formulations without incurring extra cost and time to the product development process. It is especially important to apply these technologies to new vaccines under development against diseases with high morbidity and mortality that will be used in low-resource settings, including malaria, HIV, tuberculosis, pandemic influenza, and bacterial and viral diarrheal diseases, so that introduction of these vaccines in thermostable formulations has the maximal impact.

### **Costs**

The costs involved in reformulation of existing vaccines can be significant, depending on the changes introduced. In addition to the technical development of a novel, thermostable formulation, the costs of preclinical and clinical testing required to support regulatory approval must be considered. Production of a thermostable dry powder might require an entirely new manufacturing process. Drying processes such as SD, SFD and SCFD are all scalable and continuous processes; however, it remains to be determined whether such processes will be more economical and lower cost than the well-established industry standard of lyophilization. Finally, any additional excipients required for the thermostable formulation are likely to increase the cost per dose of the vaccine.

It is difficult to ascribe a monetary value to the benefit of thermostability. Cost-effectiveness modelling of the introduction of single-dose, thermostable formulations of measles, yellow fever, BCG and DTP–hepatitis B vaccines in Cambodia, Ghana and Bangladesh found that effectiveness of all the vaccines was increased by having a thermostable format, but incremental costs also increased for three of the four vaccines [6]. Applying established WHO criteria for cost-effectiveness (interventions that cost less than three-times the average per capita income per disability adjusted life-year averted are considered cost-effective [25]), thermostable vaccines in single-dose formats were found to be cost-effective in each of the three countries according to the model used. The authors noted, however, that price considerations are critical in determining the affordability (and presumably the uptake) of thermostable vaccines at the country level [6].

**Table 3. Challenges involved in developing thermostable vaccines.**

Issue	Challenges	Consequences and solutions
<b>Regulatory</b>		
Addition of novel stabilizers, adjuvants or excipients	Novel components might be unproven in terms of safety, immunogenicity or quality of raw ingredients	Additional regulatory scrutiny might be applied; use excipients of proven safety whenever possible
Introduction of novel production processes or novel equipment	Production facilities need to comply with good manufacturing practices in order to produce material for clinical trials	Additional regulatory scrutiny might be applied
Healthy infants are the target population	The tolerance of serious adverse events in healthy infants is extremely low	Use excipients of proven safety if possible; new formulations might not be adopted
Convincing demonstration of safety will be required	Very rare, serious adverse events can be detected only in very large clinical trials	Postmarketing surveillance will be required
<b>Technical</b>		
Formulation development might be complex	There is no predictive rapid potency assay; many diseases/vaccines do not have good predictive preclinical models	Lack of preclinical models might increase amount of clinical testing needed for approval
Demonstrating clinical efficacy of reformulated product	There is still a lack of validated clinical end points and biomarkers (including assays of immune function) for many diseases	Longer, larger clinical trials with clinical end points might be needed; noninferiority trials comparing immunogenicity with existing vaccine might be possible
Reformulation of vaccines that are used in combinations	The components of combination vaccines can interact differently with each other and also with excipients	Extensive development and testing can be required, including noninferiority clinical studies
<b>Commercial &amp; intellectual property</b>		
Costs associated with developing and obtaining registration for reformulated vaccines are large and are not compatible with the low prices paid for vaccines for public-sector markets	Lack of commercial incentive for manufacturers to produce improved formulations	Procurement incentives might be required to convince vaccine manufacturers to invest
It is often difficult to quantify the problem (e.g., health and economic impact of vaccine instability) and the potential benefits of the stable vaccines	Improvements such as thermostability might not lead to a sufficient price premium to cover the development costs	Economic analyses of the impact of the stability improvement upon the whole immunization system could be useful; advocacy might be needed around both the problem and solution to proceed
Vaccine producer IP	The need to protect IP means that manufacturers are often reluctant or unable to share critical information (e.g., formulations, production methods and assays) necessary to develop improvements to vaccines outside of individual vaccine-manufacturing facilities	R&D might be limited to individual manufacturers and the pace of development driven by their interests
Technology IP	The owners of stabilization technologies must be convinced of public-sector health priorities to ensure that such technologies are made broadly available and do not adversely impact the affordability of public-sector vaccines	Organizations acting on behalf of public-sector interests can create contract mechanisms to protect IP on behalf of the public sector; advocacy might be needed around both the problem and solution to proceed

IP: Intellectual property.

**Regulatory requirements**

Changing the formulation, which sometimes involves the change of manufacturing process and delivery device and/or route of an existing, approved vaccine, requires the generation of new data to support the changes and regulatory approval for the modified product before it can be used. The degree of testing and level of scrutiny will depend upon the relevant national regulatory authority and the type and extent of change. The

type of regulatory approval required will vary from a simple notification of the change (as might be needed for changes to the container or addition of inactive excipients) to a full new biologics license application for changes made to active components or major changes to the manufacturing process. Furthermore, the exact nature of preclinical and clinical testing required and the level of regulatory scrutiny will be case-specific.

The WHO has guidelines for the stability testing of vaccines stored in the cold chain [104] and also for active pharmaceutical products stored OCC [105]. In the latter case, stability testing conditions are determined according to the climatic zone in which the product is to be used (TABLE 4). In the case of thermostable vaccines, however, these guidelines might be too simplistic. With the advent of freeze-preventive or freeze-protective formulations, it might also become necessary to test for stability at freezing temperatures. Furthermore, thermostable formulations that permit OCC storage of vaccines for part of their distribution will result in periodic temperature swings or excursions. The maximum temperature and durations that can be tolerated will need to be demonstrated and the testing required stipulated by guidelines. New guidelines from the WHO or national regulatory agencies must be created for the producers to follow in developing stable formulations.

### Opportunities & benefits associated with the use of thermostable vaccines

Several opportunities exist in the short-to-medium term to increase the use, and hence reap the benefits, of thermostable vaccines and pave the way for developing and introducing thermostable formulations of new vaccines under development.

### Exploitation of stability of existing vaccines

It is an oversimplification to think that a vaccine needs to be thermostable throughout its shelf life in order to have added value. In many cases, thermostability during final transportation and delivery stages could have great benefits. Current formulations of several vaccines are sufficiently resistant to heat damage that they could be stored, transported or used OCC for part of their shelf life. Indeed, given the consequences and frequency of freeze damage, it has been suggested that vaccines such as tetanus toxoid might be safer OCC [1].

### OCC use of hepatitis B vaccine

Most of the data relating to and supporting OCC use of vaccines have been obtained with hepatitis B vaccine. The vaccine has an expiration date of 3 years at 2–8°C, and a vaccine from one manufacturer has been shown to be stable for at least 4 years stored under those conditions, for 3 months at ambient temperature, for 1 month at 37°C and for 1 week at 45°C [26].

There is strong public-health motivation for removing hepatitis B vaccine from the cold chain. Half of the global deaths owing to hepatitis B virus infection occur in the western Pacific region. Administration of a birth dose of hepatitis B vaccine is essential to prevent mother-to-child transmission of the virus. However, approximately 30% of infants in the western Pacific region are born at home or in a health center with limited facilities, with the result that delivering the first dose of hepatitis B vaccine to infants within 72 h of birth is often limited by cold chain constraints [27].

Studies in China [27,28], Indonesia [29,30] and Vietnam [31] have compared the antibody responses in infants who received a first birth dose of monovalent hepatitis B vaccine that had been stored OCC (the second and third doses were stored at 2–8°C), with antibodies induced in infants who received all three doses of vaccine that had been stored in the cold chain. Overall, the studies involved serological analysis of more than 2500 subjects: no significant differences were seen between the seroconversion rates of infants who received an OCC birth dose of hepatitis B vaccine compared with those receiving three doses of the vaccine that had been stored in the cold chain. Studies have also demonstrated no difference in the serological response in hepatitis B virus-seronegative volunteers when vaccinated with either three doses of hepatitis B vaccine stored under normal conditions or three doses stored at 37°C for 1 week [32], or 37°C or 45°C for 1 month [26].

### OCC use of meningitis C conjugate vaccine

Menjugate® (Sanofi Pasteur MSD) is a polysaccharide conjugate vaccine composed of meningococcal C oligosaccharides conjugated to the nontoxic mutant of diphtheria toxin CRM197. The vaccine is stored and transported lyophilized and diluted prior to use with an aluminum hydroxide-containing diluent. Administration to toddlers of a single dose of Menjugate stored for 6 months at 2–8°C was compared with a single dose of vaccine that had been stored at room temperature for 6 months [33]. At the end of this period, the vaccine was still within specification and there was no difference in the safety, reactogenicity or immunogenicity in toddlers of the in-cold-chain and OCC batches, supporting OCC use of this vaccine. However, these findings will not necessarily apply to other conjugate vaccines. Ho *et al.* reported that the formulation and conjugation chemistry influenced the structural stability and immunogenicity of meningococcal C vaccines from different manufacturers, even when the same carrier protein was used [34].

**Table 4. WHO classification of climate zones and implications for stability testing.**

Zone	Climate	Measured mean annual data		Long-term stability testing conditions	
		Temperature (open air; °C)	Partial water vapor pressure (hPa)	Temperature (°C)	Relative humidity (%)
I	Temperate climate	≤15	≤11	21	45
II	Subtropical and Mediterranean	>15–22	>11 to 18	25	60
III	Hot and dry	>22	≤15	30	35
IVa	Hot and humid	>22	>15 to 27	30	65
IVb	Hot and very humid	>22	>27	30	75

Adapted from [105].



### HPV vaccines

Gardasil® (Merck) is a virus-like particle vaccine against HPV types 16, 18, 6 and 11. Using a combination of monoclonal antibody-based *in vitro* potency assays and differential scanning calorimetry the vaccine has been shown to be extremely stable, retaining over 80% of activity after 1.5 months at 37°C, and with predicted half-life estimates of 130 months or longer at temperatures up to 25°C, 18 months at 37°C and 3 months at 42°C [35].

Although the current recommendations are for storage at 2–8°C, in theory, it should be possible to obtain data to support storage for up to 3 years at 2–25°C.

### Regulatory considerations for OCC use of vaccines

The current OCC use of hepatitis B vaccines in some countries is off-label (i.e., outside the scope of the vaccine's approved label). The preclinical and clinical data suggest that other vaccines such as meningitis C and HPV vaccines may be suitable for OCC use for a limited period of the total shelf life (the vaccines would be kept at 2–8°C for most of the storage period between manufacture and use). However, off-label use is not currently supported by manufacturers and can also be confusing to healthcare workers. Suitable guidelines are therefore required for OCC use to avoid the potentially severe consequences if the vaccine loses its potency. The most appropriate way forward is for the regulatory authorities to design a regulatory process and for vaccine producers to generate stability and clinical data required to support label changes to allow OCC use. Cooperation between these key stakeholders will be critical if progress is to be made with this approach.

There are several scenarios for OCC use of heat-stable vaccines. It might be relatively straightforward to extend label claims to allow storage for months or years at ambient temperatures up to 25°C or even higher. Ambient temperatures can vary, however, and if label claims are to be extended to allow for higher temperature excursions (e.g., up to 40°C), then it will be increasingly important to monitor and control each batch or shipment to ensure that temperatures do not exceed the maximum limit. Proper use of tools such as electronic temperature recorders and VVMs will be essential. Alternatively, label claims could be obtained to recommend storage at 2–8°C but to allow temperature excursions up to 37°C for 1–6 months, which may allow OCC use during international shipment to decrease costs and prevent inadvertent freezing, and near the end of the shelf life and distribution chain, where the greatest difficulties occur with logistics and refrigeration equipment.

### Introduction of freeze-protected vaccines

Thermostable formulations that involve minimal changes to the vaccine composition or production process are likely to be adopted sooner than those involving major changes or novel excipients. The freeze-protection formulations based on polyols described previously have several properties that make them suitable for rapid adoption. The formulations appear to be broadly applicable to all vaccines and diluents containing aluminium

adjuvants. Thus, routine childhood vaccines such as DTP, hepatitis B and pneumococcal vaccines could all benefit from the technology, as well as new vaccines currently in development. The freeze-protection stabilizers have been widely used as excipients in parenteral medications and have a proven safety record. In some instances, the freeze-stable formulation can be combined with a heat-stable formulation, as demonstrated with hepatitis B vaccine. There is no intellectual property barrier for manufacturers wishing to adopt the freeze-protection technology as it has been placed in the public domain. Current efforts to reformulate and commercialize existing vaccines utilizing these freeze-protection formulations could set a precedent, paving the way for integration of the freeze-protection technology in other reformulated and new vaccines.

### Expert commentary

Reducing dependence on the cold chain by developing vaccine formulations that are resistant to damage by heat or inadvertent freezing could have great economic and health benefits by reducing vaccine wastage and preventing the health consequences of administering damaged, ineffective vaccines to infants and older recipients. Furthermore, as new vaccines are developed and introduced into immunization programs, increasing stress is placed on the capacity of the cold chain. Experience with the newly introduced rotavirus vaccines (RotaTeq® and Rotarix®) has indicated that the new vaccines require considerably more storage volume than traditional vaccines, and cold chain capacity must be planned accordingly [36]. Removal of some vaccines from the cold chain could help to compensate for the increased requirements for space. Finally, the ability to use vaccines OCC would facilitate on-time delivery of the birth dose of vaccines such as the hepatitis B vaccine and simplify vaccine delivery to remote populations and in emergency situations. It should be noted that a management system for vaccine delivery will still be essential, even where traditional cold-chain equipment is not being utilized.

The challenges and costs associated with the development of thermostable formulations of existing vaccines should not be underestimated. However, certain activities have the potential to shorten the time before the benefits of thermostability can be reaped. Some existing vaccines such as hepatitis B and HPV vaccines already have excellent heat-stability profiles. Data should be collected in an effort to support label changes for these vaccines to enable OCC use. In cases where reformulation is required, excipients that already have a history of use in the target population should be used wherever possible in order to shorten development timelines and the regulatory pathway. This approach has already produced a promising heat- and freeze-stable formulation of hepatitis B vaccine.

In developing new vaccines, consideration of stability optimization should be an essential component of the product-development process. By applying high-throughput technologies and technologies addressing specific problems (e.g., freeze sensitivity) of the vaccines in the early stages of development, vaccine producers and programs are unlikely to incur additional cost or time.

However, it should be noted that, although thermostability and thermostable formulations are the focus of this article, other factors also determine the ultimate composition and presentation of the vaccine, such as the need for preservatives or adjuvants.

### Five-year view

The next 5 years should see increasing OCC use of vaccines against hepatitis B, HPV and tetanus. Some reformulations of existing vaccines, such as the hepatitis B vaccine, with enhanced stability will be introduced into immunization programs. Ideally, promising formulation technologies currently in development will be widely used to develop stable formulations of new vaccines.

Finally, the development of thermostable formulations will ideally proceed hand-in-hand with the development of novel vaccine-delivery technologies, such as aerosols, jet injectors, patches and biodegradable implants.

### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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## Key issues

- The cold chain has been a critical component in the successful delivery of all vaccines worldwide, but it is still a vulnerable part of all immunization programs.
- Thermostable vaccines could have many benefits, including improving the effectiveness of vaccines, reducing the cold chain capacity needed to support immunization programs and enabling vaccine delivery to remote populations.
- Encouraging data have been obtained using inexpensive, generally regarded as safe excipients to produce freeze- and heat-stable formulations of some vaccines. However, the development and implementation of thermostable formulations of existing vaccines can be time-consuming and costly. Mechanisms to incentivize manufacturers to adopt these technologies are required.
- Some current vaccines are already sufficiently stable to be stored outside the cold chain (OCC) for at least part of the distribution process, facilitating the delivery of birth doses and extending coverage to remote areas. Studies to generate the stability and efficacy data to support label change allowing the use of OCCs should be encouraged.

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