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## Special Topic Commentary

## The Science is There: Key Considerations for Stabilizing Viral Vector-Based Covid-19 Vaccines



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## ABSTRACT

Once Covid-19 vaccines become available, 5–10 billion vaccine doses should be globally distributed, stored and administered. In this commentary, we discuss how this enormous challenge could be addressed for viral vector-based Covid-19 vaccines by learning from the wealth of formulation development experience gained over the years on stability issues related to live attenuated virus vaccines and viral vector vaccines for other diseases. This experience has led –over time– to major improvements on storage temperature, shelf-life and in-use stability requirements. First, we will cover work on ‘classical’ live attenuated virus vaccines as well as replication competent viral vector vaccines. Subsequently, we address replication deficient viral vector vaccines. Freeze drying and storage at 2–8 °C with a shelf life of years has become the norm. In the case of pandemics with incredibly high and urgent product demands, however, the desire for rapid and convenient distribution chains combined with short end-user storage times require that liquid formulations with shelf lives of months stored at 2–8 °C be considered. In confronting this “perfect storm” of Covid-19 vaccine stability challenges, understanding the many lessons learned from decades of development and manufacturing of live virus-based vaccines is the shortest path for finding promising and rapid solutions.

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## Introduction

Currently, different types of vaccine technologies are under development to immunize the world population against the SARS-CoV-2 virus. On October 13, 2020, one hundred ninety-nine Covid-19 vaccines were in a preclinical development phase and forty-nine in different phases of clinical development. These include candidate vaccines based on RNA, DNA, replicating and non-replicating viral vectors, inactivated and live attenuated viruses, protein subunits and virus-like particles.<sup>1</sup>

Among the promising and front-running candidate Covid-19 vaccines, one can find 57 replication competent and replication deficient viral vector-based and live attenuated virus vaccine

candidates from 11 companies.<sup>1</sup> Although not all details on storage conditions can be found in the protocols publicly available, clinical trial materials of viral vector-based Covid-19 vaccine candidates are typically transported and stored in a frozen state ( $\leq -20$  °C), unless sufficient long-term stability data has been generated to justify storage at refrigerator temperatures. By comparison, examination of currently available, commercial live attenuated virus and viral vector vaccines shows they are transported and stored within a controlled cold chain, i.e., at refrigerator (2–8 °C) or sub-zero ( $\leq -20$  °C) temperatures, depending on the type of vaccine (see Table 1 for examples). In 2005 the World Health Organization (WHO) reported a 50% wastage of vaccines, partly because of improper temperature control, e.g., inadvertent exposure to elevated temperatures or freezing of vaccines stored under refrigerator conditions.<sup>2</sup>

Globally, a one-to two-dose Covid-19 vaccination program would require about 5–10 billion vaccine doses to be manufactured, distributed, stored and administered. The logistics for storage, transportation and handling of such a high number of doses are

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**Table 1**  
Examples of Live Attenuated Virus Vaccines and Viral Vector Vaccines on the Market (Adapted from Kumru et al., 2014 and Hansen et al., 2015).<sup>6,7</sup>

Virus/Virus Strain or Viral Vector	Product Name/License Holder	Pharmaceutical Form/Administration Route	Shelf-Life and Storage Conditions	Transport, End-User Storage Conditions	In-Use Stability Conditions	Excipients	Reference
<b>Live attenuated virus vaccines</b>							
Varicella zoster virus/Oka/Merck strain	Varivax/MSD	Lyophilized powder and reconstitution liquid/s.c.	24 mo, 2–8 °C or below ( $\geq -50$ °C)	N.A.	30 min., 20–25 °C	Powder: sucrose, hydrolyzed gelatin, urea, NaCl; reconstitution liquid: WFI	32
Rotavirus/human RIX4414 of G1P[8] strain	Rotarix/GSK	Liquid/oral	Pre-filled applicator: 36 mo, 2–8 °C; Multi-dose squeezable tube: 24 mo, 2–8 °C	N.A.	Administer immediately after opening	Powder: sucrose, hydrolyzed gelatin, NaCl, potassium dihydrogen phosphate, KCl, monosodium L-glutamate, disodium phosphate, NaOH, urea; reconstitution liquid: WFI	33
Rotavirus/human reassortant with human G1, G2, G3, G4, and P[8] strains	RotaTeq/MSD	Liquid/oral	24 m at 2–8 °C	N.A.	Administer immediately after removal from refrigeration	Sucrose, sodium citrate, sodium dihydrogen phosphate, NaCl, polysorbate 80, culture media (containing inorganic salts, amino acids and vitamins), WFI	34
Rotavirus/human 116E strain	Rotavac/Bharat Biologicals	Liquid/oral	6 mo, $\leq 20$ °C <sup>a</sup>	End-user: 7 mo, 2–8 °C	6 h, 20–25 °C	Sucrose, sodium citrate, sodium phosphate monobasic, NaCl, polysorbate 80, culture medium	35,36
Rotavirus/bovine - human reassortant [G1, G2, G3, G4 and G9]	Rotasiil/Serum Institute of India	Lyophilized powder <sup>b</sup> and reconstitution liquid/oral	30 mo, < 25 °C	End-user: 18 mo, < 40 °C (preferably 2–8 °C)	6 h, 2–8 °C	Powder: Eagle's MEM, Hanks' salts, glutamine, sodium bicarbonate, sucrose, glycine; reconstitution liquid: citric acid, sodium bicarbonate, WFI	36,37
Influenza A & B subtype viruses/H1N1, H3N2, B/Massachusetts/2/2012 and B/Brisbane/60/2008 virus strains	Flumist/Medimmune	Liquid/intranasal	18 w, 2–8 °C	N.A.	Administer immediately after removal from refrigeration	Monosodium glutamate, hydrolyzed porcine gelatin, arginine, sucrose, dibasic potassium phosphate, monobasic potassium phosphate, gentamicin sulfate, EDTA	38

Adenovirus (type 4&7)/various type 4 and type 7 strains	Teva	Enteric coated tablet/oral	30 mo, 2–8 °C	N.A.	Not known	Monosodium glutamate, sucrose, D-mannose, D-fructose, dextrose, HSA, potassium phosphate, plasdone C, anhydrous lactose microcrystalline cellulose, polacrillin potassium, magnesium stearate, cellulose acetate phthalate, alcohol, acetone, castor oil, FD&C Yellow #6 aluminum lake dye	39,40
Variola virus (smallpox)/vaccinia virus	ACAM2000/Sanofi Pasteur	Lyophilized powder and reconstitution liquid; multi-dose/intradermal	60 mo, –15 °C to –25 °C	Transport: ≤ –10 °C; end-user: 18 mo, 2–8 °C	8 h, 20–25 °C; 30 d., 2–8 °C	Powder: HEPES, HSA, NaCl, mannitol; reconstitution liquid: glycerol, phenol, WFI	41
Yellow fever virus/17 D-204 strain	Stamaril/Sanofi Pasteur	Lyophilized powder and reconstitution liquid/s.c.	36 mo, 2–8 °C	N.A.	Administer immediately after reconstitution	Powder: lactose, sorbitol, L-histidine hydrochloride, L-alanine, NaCl, KCl, disodium phosphate, potassium dihydrogen phosphate, CaCl <sub>2</sub> , magnesium sulfate; reconstitution liquid: NaCl, WFI	42
Poliomyelitis virus/Sabin strains type 1&3	Polio Sabin One and Three/GSK	Suspension (multidose)/oral	24 mo, ≤ –20 °C	Transport & end user: 6 mo, 2–8 °C	Minimize exposure to ambient temperatures	Magnesium chloride, L-arginine, polysorbate 80 and WFI	43
Paramyxoviruses of genus Morbillivirus (measles), genus Rubellavirus (mumps), genus Rubivirus (rubella) <sup>c</sup>	M-M-RVAXPRO/MSD	Lyophilized powder and reconstitution liquid/s.c.	24 mo, at 2–8 °C	N.A.	8 h, 2–8 °C	Powder: sucrose, hydrolyzed gelatin (porcine), sorbitol, monosodium glutamate, sodium phosphate, sodium bicarbonate, potassium phosphate, Medium 199 with Hanks' salts, Eagle's MEM, neomycin, phenol red, HCl, NaCl; reconstitution liquid: WFI	44
<b>Replication competent viral vector vaccines</b> Zaire Ebola virus/rVSVΔG-ZEBOV-GP	Ervebo/MSD	Liquid for injection/i.m.	36 mo, –80 °C to –60 °C	End-user: 14 d, 2–8 °C	4 h, ambient temperatures	Recombinant human serum albumin, trometamol, WFI; HCl, NaCl	12

(continued on next page)

Table 1 (continued)

Virus/Virus Strain or Viral Vector	Product Name/License Holder	Pharmaceutical Form/Administration Route	Shelf-Life and Storage Conditions	Transport, End-User Storage Conditions	In-Use Stability Conditions	Excipients	Reference
Dengue virus serotypes 1, 2, 3, 4/ chimeric YF virus-dengue virus	Dengvaxia/Sanofi Pasteur	Lyophilized powder and reconstitution liquid/s.c.	36 mo, 2–8 °C	N.A.	Administer immediately after reconstitution	Powder: essential amino acids including phenylalanine, non-essential amino acids, arginine hydrochloride, sucrose, trehalose dihydrate, sorbitol, trometamol, urea, HCl, NaCl; reconstitution liquid: NaCl, WFI	45
JE virus/chimeric YF virus-JE virus	Imojev/Sanofi Pasteur	Lyophilized powder and reconstitution liquid/s.c.	36 mo, 2–8 °C	N.A.	6 h, 20–25 °C	Powder: mannitol, lactose, glutamic acid, KOH, histidine, HSA, NaCl; reconstitution liquid: NaCl, WFI	46
<b>Replication deficient viral vector vaccines</b>							
Zaire Ebola virus/ recombinant AdV-26 encoding GP of ZEBOV Mayinga strain	Zabdeno <sup>d</sup> /J&J	Suspension for injection/i.m.	48 mo, –85 °C to –55 °C	Transport: –25 °C to –15 °C; end-user: 20 mo, –25 °C to –15 °C or 8 mo, 2–8 °C	Administer immediately after removal from refrigeration	Disodium edetate, ethanol, histidine hydrochloride monohydrate, polysorbate 80, sodium chloride, sucrose, WFI, NaOH	23
Zaire Ebola virus/ recombinant modified Vaccinia Ankara Bavarian Nordic virus encoding: several strains	Mvabea <sup>d</sup> /J&J	Suspension for injection/i.m.	48 mo, –85 °C to –55 °C	Transport: –25 °C to –15 °C; end-user: 7 mo, –25 °C to –15 °C, or 1 mo, 2–8 °C	Administer immediately after removal from refrigeration	NaCl, trometamol, WFI, HCl	24

Abbreviations: AdV = adenovirus; d = days; DMEM = Dulbecco's modified Eagle's medium; EDTA = ethylenediamine tetraacetic acid; GP = glycoprotein; GSK = Glaxo Smith Kline; HEPES = 5 (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; h = hours; i.m. = intramuscular; JE = Japanese encephalitis; J&J = Johnson & Johnson; mo = months; MEM = Minimum Essential Medium; min. = minutes; MSD = Merck, Sharp & Dohme; N.A. = not applicable; s.c. = subcutaneous; rVSV = recombinant vesicular stomatitis virus; w = weeks; WFI = water for injection; YF = yellow fever; ZEBOV = Zaire ebolavirus.

<sup>a</sup> Non-frozen presentation expected in 2020, not yet pre-qualified by the World Health Organization (WHO).<sup>47</sup>

<sup>b</sup> Liquid presentation expected in 2020, not yet pre-qualified by the WHO.<sup>47</sup>

<sup>c</sup> Edmonston-Enders strain of measles virus, Jeryl Lynn strain of mumps virus, Wistar RA 27/3 strain of rubella virus.

<sup>d</sup> Used together as part of a vaccination regimen.

an enormous challenge, and success highly depends on the possibility to use existing infrastructure.<sup>3</sup> Storage at refrigerator conditions (2–8 °C) is highly preferred over frozen storage ( $\leq -20$  °C). Whereas frozen storage is frequently employed by manufacturers of vaccines for other diseases, maintaining such an extraordinary high number of units of Covid-19 vaccines at sub-zero temperatures is logistically much more challenging than under refrigerator conditions, particularly during transport and short-term storage at a distribution center and/or at the site of administration. Therefore, vaccine stability under the chosen storage condition is one of the key attributes to be considered when developing a Covid-19 vaccine and is on the WHO list for prioritization of Covid-19 vaccine candidates.<sup>4,5</sup>

In this commentary, we discuss how this stability challenge for viral vector-based Covid-19 vaccine candidates could be addressed by learning from the wealth of formulation development experience gained over the years on stability issues related to live attenuated virus vaccines and viral vector vaccines for other diseases. This experience has led –over time– to major improvements on shelf-life and storage temperature requirements, e.g., for live attenuated varicella virus-containing vaccines (Varivax) and (ProQuad),<sup>6</sup> as well as for ease of handling during transport, short-term storage at the site of administration and preparation for administration to the recipient. First, we cover work on ‘classical’ live attenuated virus vaccines as well as replication competent viral vector vaccines. Subsequently, we address replication deficient viral vector vaccines. Finally, we discuss some options for facilitating formulation development and improving stability of viral vector-based Covid-19 vaccine candidates.

### What Can We Learn From Formulating Live Attenuated Virus and Replication Competent Viral Vector Vaccines?

For live attenuated virus vaccines, the biology of the virulent, original virus has been altered so that virus virulence is minimized, while the capacity of the virus to replicate and induce the desired immune response is at least partially maintained. This replication competence is essential for the proper performance of the vaccine. Since these vaccines can mimic a natural infection by replicating *in vivo*, adjuvants (as used in inactivated virus and subunit vaccines) are not required. Live attenuated virus vaccines have demonstrated their value in preventing life-threatening infectious diseases worldwide. Examples of commercially available live attenuated virus vaccines, along with replication competent viral vector vaccines, are shown in Table 1.<sup>6,7</sup> Many of these vaccines were developed long ago, but some more recent examples are also highlighted.

Table 1 not only lists live attenuated virus and replication competent viral vector vaccine products on the market, it also provides information on the pharmaceutical form (e.g., freeze dried powder or liquid), route of administration, formulation composition, shelf life and storage conditions. Most live virus vaccines are freeze dried and stored under refrigerator conditions with shelf lives of  $\geq 18$  months. In contrast, RotaTeq and Flumist are liquid formulations and stored at 2–8 °C (shelf life 2 years and 18 weeks, respectively). Interestingly, these vaccines are administered orally and nasally, respectively, which mimics the natural route of infection. Furthermore, licensed live adenovirus vaccines are presented as tablets for oral administration and stored at 2–8 °C. Although the live attenuated oral polio and some rotavirus vaccines (i.e., Polio Sabin One and Rotavac) are stored in frozen liquid form at  $\leq -20$  °C, short-term storage for about 6 months at 2–8 °C is possible, which allows for supply under non-frozen conditions. From Table 1, one can observe that the stability profile of live attenuated and viral vector vaccines depends on the virus type (e.g., enveloped versus

non-enveloped virus) as well as the pharmaceutical dosage form (e.g., lyophilized versus liquid).

Although beyond the scope of this commentary, a brief mention of some unique challenges with formulation development of live attenuated virus and viral vector-based vaccine products, compared to protein-based drugs such as monoclonal antibodies, is provided to put into perspective the virus vaccine stability discussion below. Because of their structural complexity and the associated challenges to manufacture viral products at large scale, these preparations contain a mixture of infectious and non-infectious viruses (e.g., full and empty viral capsids).<sup>8</sup> Methods to monitor the potency of a live virus-based vaccine can include determination of viral particles or viral genomes, viral infectivity in cell-based assays, or immune responses in animals. Such assays are the cornerstone of live virus-based vaccine stability assessments. Yet, at the same time, most of these assays are labor intensive, time-consuming and highly variable, making determination of virus stability profiles more challenging. In addition, clinical determinations of the highest virus dose that is safe and the lowest dose that is efficacious must be established to elucidate the “stability window” of the extent of viral degradation that is acceptable across the shelf life of the vaccine product.<sup>6</sup> Therefore, a scientific understanding of the quality/quantity of viral preparations, clinical determinations of dose levels, and an analytical testing strategy of potency are interrelated key factors to be considered during formulation development of stable live virus-based vaccine products.

### Optimizing Stability of Liquid Formulations

How are excipients being used for virus stabilization and formulation purposes? Burke et al. (1999) provide a comprehensive evaluation of pharmaceutical excipients found in live attenuated virus vaccine products.<sup>9</sup> In addition, extensive information on viral potency losses under different storage conditions, in (frozen) liquid or freeze dried form, is provided. Typically, no reasons are given why these –often highly complex mixtures of excipients– are chosen, other than empirical observation of improvements in virus stability. More systematic formulation development work on the influence of excipients is becoming more common. For example, Evans et al. (2004) developed stable liquid formulations of a candidate adenovirus type-5 (AdV-5) vector vaccine by evaluating a series of excipient optimization schemes based on known/proposed mechanisms of virus inactivation.<sup>10</sup> Schlehuber et al. (2011) performed a high-throughput formulation screening, testing over 11,000 unique liquid formulations of measles virus which was genetically modified to encode the gene for a green fluorescent protein for virus replication detection.<sup>11</sup> Among the best-performing formulations in this work were those consisting of a limited number of excipients, as typically used for stabilizing recombinant therapeutic proteins. However, in contrast to the modern therapeutic protein formulations, these live virus vaccine formulations often (still) contain macromolecular excipients (e.g., proteins, peptides, polymers). The selection of only a few well-characterized excipients in the viral vector-based Ebola vaccine Ervebo, approved in 2019, exemplifies this trend away from multi-excipient, complex formulations typically used for live attenuated vaccines as described by Burke et al.<sup>9,12</sup>

### And What About Freeze Drying?

Hansen et al. (2015) reviewed existing literature on the mechanisms by which cryo- and lyo-protectants stabilize live viruses as well as the effects of freeze drying conditions (such as pre-cooling, freezing rate, annealing, primary and secondary drying conditions)

on virus potency.<sup>7</sup> Burke et al. (1999) and Tlaxca et al. (2015) have also reviewed the composition of various lyophilized live virus vaccine formulations, including veterinary vaccines.<sup>9,13</sup> Data on actual potency loss of live attenuated virus vaccine formulations upon freeze drying is available: several researchers have reported potency losses ranging between 0 and  $3.6\log_{10}$ , but systematic excipient optimization studies—as with the liquid formulations reported above—have not been as widely described.<sup>9,14–20</sup> In one recent report, Patel et al. (2018) developed lyophilized formulations of a live dengue vaccine candidate using a combination of empirical screening and design of experiment (DoE) approaches.<sup>20</sup>

Because of the cost and limited manufacturing capacity for lyophilization of sterile vaccine dosage forms, there is ongoing interest in alternative drying strategies, such as foam drying, spray drying and evaporative drying. For example, the stability of a dried live attenuated influenza vaccine was compared after lyophilization, spray drying and foam drying and it was determined that foam drying provided an order of magnitude improvement in virus stability.<sup>21</sup> Another alternative approach is to dry vaccine candidates onto dissolvable microneedles by evaporative drying, which allows for delivery via a skin patch without the need for reconstitution of the dried vaccine prior to administration. For example, live measles and rubella combination vaccines have been evaluated in such systems in preclinical models with planned clinical trials to be initiated.<sup>22</sup>

### What Can We Learn From Formulating Replication Deficient Viral Vector Vaccines?

Replication deficient viral vector vaccines consist of a viral vector engineered by molecular biological means so that it cannot replicate, but upon administration and cell infection/penetration, the encoded antigen of interest is expressed. At present, two non-replicating viral vector vaccines (AdV-26 and modified Vaccinia Ankara vectors) against Ebola have been approved by the European Medicines Agency: Zabdeno (2020) and Mvabea (2020), which are used consecutively in one vaccination protocol.<sup>23,24</sup> As shown in Table 1, both formulations consist of a limited number of well-defined excipients and both liquid formulations must be stored frozen at about  $-70\text{ }^{\circ}\text{C}$  for long-term storage; however, at  $2\text{--}8\text{ }^{\circ}\text{C}$ , the products are stable for  $\geq 1$  month. This may allow shipment and storage on site under refrigerator conditions before administration.

The Oxford/Astra Zeneca (ChAdOx1 nCoV-19) and Johnson & Johnson (J&J) (Ad26.COV2.S) ongoing clinical phase III trials to assess protection against Covid-19 both use adenoviral vector vaccines that are stored in frozen liquid form ( $\leq -20\text{ }^{\circ}\text{C}$ ).<sup>25,26</sup> However, short-term storage under non-frozen ( $2\text{--}8\text{ }^{\circ}\text{C}$ ) conditions is possible for both products.<sup>25,26</sup> The ChAdOx1 nCoV-19 vaccine is formulated with a limited number of standard excipients (i.e., sucrose, histidine, NaCl, MgCl<sub>2</sub>, polysorbate 80, EDTA and ethanol).<sup>25</sup> Zabdeno, one of the two J&J vaccines against Ebola virus that use the same Ad26 viral vector technology, is formulated as a liquid presentation with stability of 8 months at  $2\text{--}8\text{ }^{\circ}\text{C}$  (Table 1).<sup>23</sup> A freeze dried Chinese AdV-5 vector-based Ebola vaccine (Ad5-EBOV) was approved in China in 2017 and can be stored at  $2\text{--}8\text{ }^{\circ}\text{C}$  for 12 months.<sup>27</sup> Based on the same vector technology, a Covid-19 vaccine candidate has been developed, which is now—supplied in liquid form—tested in Phase II clinical trials.<sup>28</sup> Here we see a development towards a new practice where the vaccine is stable for sufficient periods of time at  $2\text{--}8\text{ }^{\circ}\text{C}$  to cover the short storage times that can be expected in emergency situations such as the Covid-19 pandemic.<sup>28,29</sup> According to the clinical protocol, long-term real time, accelerated and stress stability studies on the product are ongoing at  $2\text{--}8\text{ }^{\circ}\text{C}$  (30 months),  $23\text{--}27\text{ }^{\circ}\text{C}$  (6 months), and  $35\text{--}39\text{ }^{\circ}\text{C}$  (8 weeks), respectively, to support an eventual shelf-

life claim of years under refrigerator conditions. These examples show that using the same viral vector construct across multiple vaccine candidates can speed up formulation development by leveraging from prior experiences. For example, product and process knowledge for related viral products and their corresponding analytical methodologies (collectively referred to as a “platform technology”) can allow development milestones to be reached faster than *de novo* efforts. Nonetheless, irrespective of the value of the time saved to reach these milestones, the quality, safety, and efficacy of each new viral vaccine candidate must be established based on robust data from actual manufacturing campaigns and clinical trials.

Literature on the rational formulation design of stable replication deficient viral vector vaccines is scarce. Kumru et al. (2018) published on the stability of two engineered lentiviral vector vaccines after storage at  $4\text{ }^{\circ}\text{C}$  and freeze-thawing, as measured by both viral genome assays (PCR) and physical counting of viral particles (nanoparticle tracking analysis); they screened a wide variety of excipient classes and types from a library of 50 excipients.<sup>30</sup> Reinauer et al. (2020) used a DoE approach with iterative optimization rounds and they reported significant enhancement of the stability of liquid, adenoviral vector vaccine formulations following such an algorithm.<sup>31</sup>

### Final Considerations

The urgent need for Covid-19 vaccines to fight the world-wide pandemic has led to the rapid development of first-generation vaccine candidates that may be less than ideal from a vaccine stability and distribution perspective. In the case of viral vector vaccine candidates, formulation challenges outlined in this commentary can be the focus for development of next-generation vaccine candidates as well as second-generation formulations of the lead vaccine candidates currently in late-phase clinical trials. Improved formulations of virus-based Covid-19 vaccines will lead to better stability profiles (e.g., extending shelf life and transitioning from freezer to refrigerator storage) as well as lowering costs and facilitating widespread distribution (e.g., manufacturing of low-cost, widely available, and conveniently administered vaccine dosage forms). Based on these considerations, the following suggestions to facilitate formulation development of viral vector-based Covid-19 vaccine candidates are provided:

- When one is charged to develop a formulation for a viral vector-based Covid-19 vaccine candidate, previous experience with formulation and stabilization of live attenuated virus and replication competent viral vector vaccines, as well as the recently approved replication deficient viral vector vaccines, will provide important guidance. In addition, the wealth of information and physicochemical insights regarding the design of stable formulations for biotherapeutics should also be explored.
- Virus stability profiles during storage and administration highly depend on the type of virus. For instance, non-enveloped viruses such as adenoviruses are more stable than enveloped viruses such as measles viruses. This may be an important consideration when assessing different viral vector candidates for vaccine development to fight a pandemic.
- With advances in formulation science, highly complex formulations used for older viral vaccines, often with poorly defined excipients, can evolve into more well-defined formulations based on a smaller number of globally accepted, pharmacopeia-grade excipients, very much like those used for formulations of recombinant therapeutic proteins. Such formulations offer the ability to lower costs and improve manufacturing capacity. To this end, multi-dose formulations (e.g., 5–10 doses per vial)



would further lower costs and increase availability, although their development may be challenging since they typically require the addition of preservatives (to ensure sterility during multiple withdrawals from a single vial), which may be incompatible with live virus and viral vector vaccines during long-term storage. The addition of preservatives to the diluent used to reconstitute a lyophilized vaccine upon administration is one approach to be considered as described for the smallpox (vaccinia virus) vaccine in national stockpiles (see Table 1).

- Freeze drying of live attenuated virus and viral vector vaccine formulations to avoid sub-zero ( $\leq -20$  °C) storage conditions and allowing storage at 2–8 °C has become the standard for such vaccines for other diseases than Covid-19. Is there a competitive advantage for freeze dried Covid-19 vaccines that can be stored under refrigerator conditions compared to frozen liquid products? Yes, however, freeze drying is more expensive and the huge number of product units needed in this Covid-9 pandemic may cause production problems. In addition, the costs and complexity to the health care provider to store and prepare the lyophilized product for administration, using a separately supplied diluent and related reconstitution procedure, also have to be factored in. In contrast, liquid formulations of Covid-19 vaccines are more economical to produce, but may need to be stored in a freezer which adds to the cost and complicates transport and end-user handling. As mentioned above, in this Covid-19 pandemic, storing liquid formulations at 2–8 °C for a limited number of months may be the short-term solution<sup>28,29</sup>: fast distribution and fast administration are key words when this option is chosen. It is also possible that different regions will be at different stages of pandemic control at different times, and there may be room for multiple approaches for a given vaccine to meet this global pandemic need. In this context, the extended controlled temperature chain (ECTC) initiative of the WHO is of interest, which allows a vaccine to be kept at temperatures outside of the traditional cold chain of 2–8 °C for a limited period of time under monitored and controlled conditions.<sup>48</sup> This approach has recently been evaluated for storage of a live attenuated measles vaccine.<sup>49</sup>
- The number of publications dealing with mechanistic and systematic approaches to formulation and stabilization of live attenuated virus and viral vector-based vaccine products is low and is an important area of future research. Better insights into physicochemical mechanisms of virus potency losses, combined with rational approaches to minimize their occurrences, would be very helpful in guiding improved vaccine shelf life that may even lead to abandoning ‘cold chain’ requirements. This would dramatically lower logistic barriers and thereby improve patient access and vaccine coverage as well as decrease product wastage and costs. To this end, the development of optimized viral vector-based Covid-19 (as well as other) vaccine formulations benefit substantially with availability of high-throughput, stability-indicating analytical testing strategies of potency, especially those which correlate to clinical outcome. Such assays are easier to establish when a novel vaccine candidate is based on a previously established “platform” vaccine technology, since such test methods can more readily be adapted from already established procedures.

## References

1. Covid-19 tracker, London School of Hygiene & Tropical Medicine. [https://vac-lshtm.shinyapps.io/ncov\\_vaccine\\_landscape/](https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/). Accessed October 27, 2020.
2. Monitoring Vaccine Wastage at Country Level, WHO/V&B/03.18 Rev.1; 2005. [https://apps.who.int/iris/bitstream/handle/10665/68463/WHO\\_VB\\_03.18.Rev.1\\_eng.pdf?sequence=1&isAllowed=y](https://apps.who.int/iris/bitstream/handle/10665/68463/WHO_VB_03.18.Rev.1_eng.pdf?sequence=1&isAllowed=y). Accessed October 27, 2020.
3. Covid-19 vaccine delivery faces problems, Warns DHL. Financial Times. <https://www.ft.com/content/52d40ce8-4ff6-4068-8c48-b98fa0f7740b>. Accessed October 27, 2020.
4. WHO Target Product Profiles for COVID-19 vaccines. <https://www.who.int/publications/m/item/who-target-product-profiles-for-covid-19-vaccines>. Accessed October 27, 2020.
5. Criteria for COVID-19 vaccine prioritization. <https://www.who.int/publications/m/item/criteria-for-covid-19-vaccine-prioritization>. Accessed October 27, 2020.
6. Kumru OS, Joshi SB, Smith DE, Middaugh CR, Prusik T, Volkin DB. Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. *Biologicals*. 2014;42:237-259.
7. Hansen LJJ, Daoussi R, Vervaeet C, Remon J-P, De Beer TRM. Freeze-drying of live virus vaccines: a review. *Vaccine*. 2015;33:5507-5519.
8. Fedosyuk S, Merritt T, Peralta-Alvarez MP, et al. Simian adenovirus vector production for early-phase clinical trials: a simple method applicable to multiple serotypes and using entirely disposable product-contact components. *Vaccine*. 2019;37(47):6951-6961.
9. Burke CJ, Hsu T-A, Volkin DB. Formulation, stability, and delivery of live attenuated vaccines for human use. *Crit Rev Ther Drug Carrier Syst*. 1999;16(1):1-83.
10. Evans RK, Nawrocki DK, Isopi LA, et al. Development of stable liquid formulations for adenovirus-based vaccines. *J Pharm Sci*. 2004;93:2458-2475.
11. Schlehuber LD, McFadyena IJ, Shu J, et al. Towards ambient temperature-stable vaccines: the identification of thermally stabilizing liquid formulations for measles virus using an innovative high-throughput infectivity assay. *Vaccine*. 2011;29:5031-5039.
12. Ervebo summary of product characteristics. [https://www.ema.europa.eu/en/documents/product-information/ervebo-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/ervebo-epar-product-information_en.pdf). Accessed October 27, 2020.
13. Tlaxca JL, Ellis S, Remmele Jr RL. Live attenuated and inactivated viral vaccine formulation and nasal delivery: potential and challenges. *Adv Drug Deliv Rev*. 2015;93:56-78.
14. Kang MS, Jang H, Kim MC, et al. Development of a stabilizer for lyophilization of an attenuated duck viral hepatitis vaccine. *Poultry Sci*. 2010;89:1167-1170.
15. Zhai S, Hansen RK, Taylor R, Skepper JN, Sanches R, Slater NKH. Effect of freezing rates and excipients on the infectivity of a live viral vaccine during lyophilization. *Biotechnol Prog*. 2004;20:1113-1120.
16. Ferris NP, Philpot RM, Oxtoby JM, Armstrong RM. Freeze-drying foot-and-mouth disease virus antigens. I. Infectivity studies. *J Virol Methods*. 1990;29:43-52.
17. Shokri S, Shahkarami MK, Shafiyi A, Mohammadi A, Esna-ashari F, Hamtab A. Evaluation of the thermal stability of live-attenuated rubella vaccine (Takahashi strain) formulated and lyophilized in different stabilizers. *J Virol Methods*. 2019;264:18-22.
18. Sood DK, Aggarwal RK, Sharma SB, Sokhey J, Singh H. Study on the stability of 17D-204 yellow fever vaccine before and after stabilization. *Vaccine*. 1993;11:1124-1128.
19. Naik SP, Zade JK, Sabale RN, et al. Stability of heat stable, live attenuated rotavirus vaccine (ROTASIL). *Vaccine*. 2017;35:2962-2969.
20. Patel A, Erb SM, Strange L, et al. Combined semi-empirical screening and design of experiments (DOE) approach to identify candidate formulations of a lyophilized live attenuated tetraivalent viral vaccine candidate. *Vaccine*. 2018;36:3169-3179.
21. Lovalenti PM, Anderl J, Yee L, et al. Stabilization of live attenuated influenza vaccines by freeze drying, spray drying, and foam drying. *Pharm Res (N Y)*. 2016;33:1144-1160.
22. Prausnitz MR, Goodson JL, Rota PA, Orenstein WA. A microneedle patch for measles and rubella vaccination: a game changer for achieving elimination. *Curr Opin Virol*. 2020;41:68-76.
23. *Zabdeno Summary of Product Characteristics*; 2020. [https://www.ema.europa.eu/en/documents/product-information/zabdeno-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/zabdeno-epar-product-information_en.pdf). Accessed October 27, 2020.
24. Mvabea summary of product characteristics. [https://www.ema.europa.eu/en/documents/product-information/mvabea-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/mvabea-epar-product-information_en.pdf). Accessed October 27, 2020.
25. Clinical Protocol: A Phase III randomized, double-blind, placebo-controlled multicenter study in adults to determine the safety, efficacy, and immunogenicity of AZD1222, a non-replicating ChAdOx1 vector vaccine, for the prevention of COVID-19. [https://s3.amazonaws.com/ctr-med-7111/D8110C00001/52bec400-80f6-4c1b-8791-0483923d0867/c8070a4e-6a9d-46f9-8c32-cece903592b9/D8110C00001\\_CSP-v2.pdf](https://s3.amazonaws.com/ctr-med-7111/D8110C00001/52bec400-80f6-4c1b-8791-0483923d0867/c8070a4e-6a9d-46f9-8c32-cece903592b9/D8110C00001_CSP-v2.pdf). Accessed October 27, 2020.
26. A randomized, double-blind, placebo-controlled Phase 3 study to assess the efficacy and safety of Ad26.COV2.S for the Prevention of SARS-CoV-2-Mediated COVID-19 in adults aged 18 years and older. <https://www.njn.com/coronavirus/covid-19-phase-3-study-clinical-protocol>. Accessed October 27, 2020.
27. Ebola vaccines – Background paper for SAGE deliberations. Overview of candidate Ebola vaccines as of August 19, 2019. [https://www.who.int/immunization/sage/meetings/2019/october/6\\_Ebola\\_Candidate\\_Vaccines\\_19-09-19.pdf](https://www.who.int/immunization/sage/meetings/2019/october/6_Ebola_Candidate_Vaccines_19-09-19.pdf). Accessed October 27, 2020.
28. Zhu FC, Li YH, Guan XH, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet*. 2020;395:1845-1854.
29. Clinical protocol: a randomized, double-blind, placebo-controlled phase II clinical trial of the recombinant novel coronavirus vaccine (adenovirus type 5



- vector) in healthy adults aged 18 years and above in China. <http://www.jsdc.cn/jkfw/kygz/202007/P020200720346693837711.pdf>. Accessed October 27, 2020.
30. Kumru OS, Wang Y, Gombotz CWR, et al. Physical characterization and stabilization of a lentiviral vector against adsorption and freeze-thaw. *J Pharm Sci.* 2018;107:2764-2774.
  31. Reinauer EB, Grosso SS, Henz SR, et al. Algorithm-based liquid formulation development including a DoE concept predicts long-term viral vector stability. *J Pharm Sci.* 2020;109:818-829.
  32. Varivax summary of product characteristics. [https://mri.cts-mrp.eu/human/downloads/IT\\_H\\_0114\\_001\\_FinalPI.pdf](https://mri.cts-mrp.eu/human/downloads/IT_H_0114_001_FinalPI.pdf). Accessed October 27, 2020.
  33. Rotarix Product Details GSK, UK. <https://www.medicines.org.uk/emc/product/201/smpc#gref>. Accessed October 27, 2020.
  34. RotaTeq summary of product characteristics. [https://www.ema.europa.eu/en/documents/product-information/rotateq-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/rotateq-epar-product-information_en.pdf). Accessed October 27, 2020.
  35. Rotavac WHO training package. <https://www.who.int/immunization/diseases/rotavirus/rotavac/en/>. Accessed October 27, 2020.
  36. Rotavirus Vaccine Information Sheet WHO; 2018. [https://www.who.int/vaccine\\_safety/initiative/tools/Rotavirus\\_vaccine\\_rates\\_information\\_sheet\\_0618.pdf](https://www.who.int/vaccine_safety/initiative/tools/Rotavirus_vaccine_rates_information_sheet_0618.pdf). Accessed October 27, 2020.
  37. Rotasiil WHO one dose training package. [https://www.who.int/immunization/diseases/rotavirus/rotasiil\\_1dose/en/](https://www.who.int/immunization/diseases/rotavirus/rotasiil_1dose/en/). Accessed October 27, 2020.
  38. Flumist Prescribing Information FDA. <https://www.fda.gov/media/120689/download>. Accessed October 27, 2020.
  39. Adenovirus Type 4 and Type 7 vaccine, live, oral enteric coated tablets prescribing information FDA. <https://www.fda.gov/media/80211/download>. Accessed October 27, 2020.
  40. Gray GC, Erdman DD. Adenovirus Vaccines. *Plotkin's Vaccines*; 2018:121-133.e8. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7151885/pdf/main.pdf>. Accessed October 27, 2020.
  41. ACAM2000 Prescribing Information FDA. <https://www.fda.gov/media/75792/download>. Accessed October 27, 2020.
  42. Stamaril Summary of Product Characteristics. [https://www.ema.europa.eu/en/documents/referral/stamaril-article-30-referral-annex-i-ii-iii\\_en.pdf](https://www.ema.europa.eu/en/documents/referral/stamaril-article-30-referral-annex-i-ii-iii_en.pdf). Accessed October 27, 2020.
  43. Polio Sabin One and Three (Oral) WHO Package Insert. [https://www.who.int/immunization\\_standards/vaccine\\_quality/OPV\\_bivalent\\_GSK\\_WHO\\_package\\_insert\\_text\\_english.pdf](https://www.who.int/immunization_standards/vaccine_quality/OPV_bivalent_GSK_WHO_package_insert_text_english.pdf). Accessed October 27, 2020.
  44. M-M-RVAXPRO. Summary of product characteristics. [https://www.ema.europa.eu/en/documents/product-information/m-m-rvaxpro-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/m-m-rvaxpro-epar-product-information_en.pdf). Accessed October 27, 2020.
  45. Dengvaxia summary of product characteristics. [https://www.ema.europa.eu/en/documents/product-information/dengvaxia-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/dengvaxia-epar-product-information_en.pdf). Accessed October 27, 2020.
  46. Japanese Encephalitis Vaccine (Live) (4 dose vial) WHO package insert. [https://www.who.int/immunization\\_standards/vaccine\\_quality/PQ\\_277\\_JE\\_4dose\\_SP\\_PI.pdf?ua=1](https://www.who.int/immunization_standards/vaccine_quality/PQ_277_JE_4dose_SP_PI.pdf?ua=1). Accessed October 27, 2020.
  47. Burke RM, Tate JE, Kirkwood CD, Steele AD, Parashar UD. Current and new rotavirus vaccines. *Infect Dis.* 2019;32:435-444.
  48. Extended Controlled Temperature Conditions (ECTC). <https://www.who.int/biologicals/areas/vaccines/ectc/en/>. Accessed November 15, 2020.
  49. Juan-Giner A, Alsalhani A, Panunzi I, Lambert V, Van Herp M, Gairola S. Evaluation of the stability of measles vaccine out of the cold chain under extended controlled temperature conditions. *Vaccine.* 2020;38:2473-2477.