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REVIEW

Viral vaccine stabilizers: status and trends

F. M. C. CARDOSO[1], D. PETROVAJOVÁ[1], T. HORŇÁKOVÁ[1,2]

[1]Research and Development Department, Imuna Pharm a.s., Jarková 17, 082 22 Šarišské Michaľany, Slovak Republic;
[2]Olomouc University Social Health Institute (OUSHI), Olomouc, Czech Republic

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Summary. – Vaccine stability is a key factor to preserve vaccine potency and efficiency, as its potency decays over time and during temperature changes. The choice of stabilizers for viral vaccine formulation depends mainly on the vaccine type. More specifically, the choice is determined by the properties and structure of the active pharmaceutical ingredient or viral antigen(s) in the vaccine. In this review, we analyze key formulation components in different vaccine types. We discuss some of the major driving forces in the improvement of vaccine thermostability: increasing demand for cost-effective production of thermostable vaccine with lower dependency on cold chain, stricter regulatory policies for animal-origin materials, and the return of the research investment from the industry point of view. Moreover, we provide an overview of existing licensed viral vaccine types, including their production platform, presentation, delivery route, known stabilizers content and available thermostability data. In addition, we compare the data of licensed vaccines to published experimental vaccines, in order to discuss the current trends in vaccine stabilizers development.

Keywords: vaccines; viruses; stabilizers; thermostability; protein components; formulation

Contents:

1. Introduction

It is widely accepted that vaccination has been one of the most successful medicine tools. By the first half of the 20th century, newly developed cell culture techniques allowed *in vitro* pathogen passaging, resulting in the attenuation of several medically relevant infectious diseases agents. Throughout the last 200 years, several technology waves have helped to define the most important milestones in vaccine history, considering five past revolutions in vaccinology: virus attenuation, virus inactivation, cell culturing of viruses, genetic engineering and induction of cell-mediated immunity by vaccine (Plotkin, 2005). In the 21st century, genetically engineered vaccines, reverse vaccinology and potent immunoselective adjuvants are the main vaccine innovative driving forces. It is expected that one or several of these innovations would become a market game-changer and will contribute to the next vaccine revolution. The development of these innovative technologies can be considered as a reaction to: progressively stricter safety concerns of regulatory agencies; need for more potent and cost-effective vaccines and increase the effectiveness of the vaccine immunization coverage.

The viral antigen(s) or active pharmaceutical ingredient (API) is the immunogenic component of the vaccine. Until

E-mail: miguel.lopez.cardoso@gmail.com; phone: +56-945012852.
Abbreviations: API = active pharmaceutical ingredient; GRAS = generally regarded as safe; HSA = human serum albumin; IN = inactivated vaccines; LAV = live-attenuated vaccines; SUB = subunit or recombinant vaccines; VLP = virus-like particles

recently, the API structural analysis prior to stabilizers formulation selection has not been used in the process of the vaccine formulation. Historically, vaccine formulations have been relying heavily on empirical approaches, resulting mostly in successful vaccines, but with limited thermostability. Nowadays, an important part of the vaccination coverage effectiveness relies on the maintenance of an expensive cold chain systems used to ensure proper vaccine storage, distribution and administration. However, the constant streaming of new scientific information regarding virus structure, infection and immune system regulation is making the selection of stabilizers in vaccine development more scientifically-driven. In line with this tendency, new technological advancements based in time-saving high-throughput technologies (Schlehuber *et al.*, 2011; Walter *et al.*, 2012) and rational and systematic antigen structure-based vaccine formulation (Morefield, 2011) represent major current trends in vaccine development. In this review, we discuss recent scientific, regulatory and economical constrains and their impact on current viral vaccines stabilizers development. We further focus on the correlation of the API characteristics with components of the licensed vaccines, to compare them with components of publically available experimental vaccine data and to analyze current trends in vaccine stabilizers development.

2. Viral vaccine types and their stability

Based on current vaccine stabilizers data, the more complex is the API the more complex tends to be its vaccine formulation. Notably, viral vaccines with their complex structure and functioning, expensive production and high safety risks represent a particular challenge to the pharma industry and regulatory agencies worldwide. There are three different viral vaccine types: the live attenuated vaccines (LAV), the inactivated vaccines (IN), and the recombinant or subunit vaccines (SUB). As their API structural characteristics are remarkably different, API complexity correlates with the vaccine variable stability.

The LAV were among the first vaccines ever used in humans (*e.g.* smallpox, rabies, tuberculosis, yellow fever; Plotkin, 2014), and in the first half of the 20th century vaccine attenuation resulted in a significant decrease in pathogenicity. During the second half of the 20th century, several relevant viral LAV had a breakthrough success, including: polio, measles, mumps, rubella, adenovirus, varicella, rotavirus and influenza virus vaccines (Plotkin, 2014). In most cases, viral LAV replication within the host is as potent as the wild type virus, without the onset of disease symptoms. LAV potency correlates directly with their replicative potential, resulting in a sustained immune response, including innate response (inflammation), but most importantly a strong adaptive response (antigen-specific antibody production and a strong cellular-mediated response). The LAV has been historically known for their efficacy, but structural changes induced by external factors impact their potency, with the temperature as the most critical. Depending on the structure of viral capsid outer layer, the LAV virus capsid can be either enveloped (*i.e.* containing a host lipid membrane: measles, mumps, influenza, smallpox, rabies viruses) or capsid protein (*i.e.* geometric arrangement of copies of one or more viral proteins: polio virus, adenovirus, rotavirus, papilloma virus). Independently of their API complexity and structural architecture of their capsid, most LAV are thermosensitive and require rich formulations to create a complex glassy matrix to prevent lipid membrane hydration and protein aggregation (Alcock *et al.*, 2010). Based on available information, LAV formulations consist of buffers (*e.g.* phosphate, citrate) including one or two of the following stabilizers types: i) protein components (*e.g.* peptides, amino acids, human serum albumin, lactoalbumin, gelatin, polygeline) or ii) sugar and sugar alcohols (*e.g.* sucrose, trehalose, sorbitol, mannitol, lactose) (Table 1). Therefore, licensed LAV formulation is mostly limited to solid state (lyophilization), with a few exceptions. Since LAV immunogenicity relies on replicative component, the major goal of LAV technological development is the optimization of its stabilizers composition.

The IN are live vaccines inactivated by virion exposition to a cross-linking agent that does not affect their antigenicity. Among the most commonly used commercial vaccines inactivating agents are the formaldehyde (as formalin) and β-propiolactone (Perrin and Morgeaux, 1995). Due to their inactivation, IN vaccines do not have a replicative potential (a desirable vaccine safety feature for regulatory authorities), and the immunological response triggered is less potent in comparison to LAV. Consequently, in most of the inactivated IN vaccines, effectiveness relies on the wide use of adjuvants in their formulation, and/or subsequent boosting dose(s). Since IN vaccines require an adjuvant, it is of the outmost importance to preserve the desired physicochemical properties of the API-adjuvant complex, in order to elicit the desired immune response. Nowadays, the selection of the

Table 1. Different classes of "generally regarded as safe" (GRAS) excipients and examples found in licensed viral vaccines

Excipient class	Examples
Amino acids	arginine, aspartate, glycine, glutamate, histidine, lysine, proline
Antioxidants	ascorbic acid, EDTA, malic acid
Proteins	human albumin, gelatin
Sugars/polyols	dextrose, glycerol, lactose, mannitol, myo-inositol, sorbitol, sucrose, trehalose
Surfactants	pluronic, tween, polysorbate

Table 2. Licensed adjuvants used in human inactivated (IN) vaccines

Adjuvant	Representative (year when licensed)	Components	Vaccines (disease)
Mineral salts	Aluminium salts (1924)	Aluminium phosphate or hydroxide	Anflu (pandeminc influenza), Ixiaro (Japanase encephalitis), Havrix (rabies), Engerix (hepatitis B), Gardasil (human papilloma)
Oil-in-water emulsion	MF59 (Novartis; 1997), ASO3 (GlaxoSmithKline; 2009)	Squalene-based emulsions particles, stabilized with detergents (polysorbate 80 (Tween 80, Span 85)	Fluad (seasonal influenza), Aflunov/Prepandrix (pre-pandemic influenza), Focetria/Pandremix (pandemic influenza)
Liposomes	Virosomes (Berna Biotech; 2000)	Lipids, viral proteins (e.g. hemagglutinin)	Inflexal (seasonal influenza) Epaxal (hepatitis A)
Alum-absorbed TLR4 agonist	AS04* (GlaxoSmithKline; 2005)	Aluminium hydroxide, MPL	Fendrix (hepatitis B) Cervarix (human papilloma virus)
Oil-in-water emulsion	AF03 (Sanofi Pasteur; 2010, withdraw in 2011)	Squalene, Montane 80, Eumulgin B1 PH	Humenza (pandemic influenza)

licensed adjuvants is surprisingly limited. Aluminium salts (aluminium phosphate or hydroxide) are by far the most widely used adjuvants for more than 70 years (Clapp *et al*., 2011). However, the unmet need for more selective and potent adjuvants is fueling an intensive research of vaccine adjuvants and is expected to lead to new generation of inactivated vaccines (Garcia and De Sanctis, 2014, Mohan *et al*., 2013). A brief overview of current licensed adjuvants is given in Table 2, but new promising alternatives to licensed adjuvants are also reported, including the two main groups, TLR antagonists (Fujita *et al*., 2013) and colloidal carriers (Beg *et al*., 2013). Because of higher stability of IN vaccines compared with LAV, licensed IN vaccines are available also in liquid form (Supplementary Table S3). When IN vaccines have non aluminium-based adjuvants, the delivery route can be more versatile. For example, the oil-in water preparations (emulsions) and virosomes-based approaches are presented in both, lyophilized and liquid licensed vaccine formulations (Supplementary Table S3).

The genetic engineering is widely used for SUB vaccine production and those vaccines are considered the safest and most promising of the current vaccine types. Viral recombinant or SUB vaccines consist of immunogenic viral proteins and/or polysaccharides, generally from the exterior of the virus capsid. As highly purified proteins tend to be thermostable**,** by reducing the amount of stabilizers within the formulation, the SUB vaccine is an attractive alternative to IN or LAV vaccines. For example, the SUB vaccine FluBlok® has no additives (Supplementary Table S3). SUB vaccines improved thermostability allows for a variety of innovative delivery routes. On the negative side, SUB vaccines require several boosts for complete protection, which poses an extra vaccine distribution, storage and logistical challenges. Interestingly, there are two relatively recent innovations in SUB vaccines that can target these disadvantages, representing current research trends in SUB vaccine field. The first innovation is the capacity of some viral proteins to self-assembly into virus like particles (VLP) with more potent immunogenic potential than the single soluble protein. This is achieved by the multi-epitopic nature of VLP, increasing the pathogen recognition by the immune response and inducing strong humoral and cellular responses. One of the main advantages of VLPs is the relatively easy microbial production platform and the possibility of cell-free systems (Rodriguez-Limas, 2013). Notably, licensed vaccines against the human papilloma virus and associated cancer confirm the VLP as an effective platform (Gardasil® and Cervarix®) (Supplementary Table S3). The second innovation is the reverse engineering, a technique that implies the sequencing of the virus, synthesizing several of its antigens, inducing immune response *in vivo*, and subsequently developing the most immunogenic antigens as vaccine (Rappuoli, 2000).

3. Vaccine excipients and safety regulations

Vaccine excipients comprise all chemical compounds used in the production of vaccines, including: the suspending fluid (*e.g.* sterile water, saline buffer or fluids containing protein), preservatives (*e.g.* antibiotics, thiomersal), stabilizers and adjuvants that contribute to the vaccine's efficiency, as well as traces of the culture material (*e.g.* media, FBS). Vaccine composition varies from one product to another, and different vaccines with the same API can have substantially different formulation composition attributed to production processes. All these aspects represent critical manufacturer know-how and the vaccine stabilizer composition can be protected as intellectual property. Depending on the vaccine type, the inclusion of stabilizing agents can be crucial for API stability. Generally, most of the stabilizers found in commercial viral vaccines are considered as generally regarded as safe (GRAS) compounds (Table 1). The main advantage of using GRAS

as vaccine stabilizers is the traceability of materials and time saving during the vaccine qualification and certification, by national and international regulatory authorities. One of the biggest challenges for vaccine manufacturers is the constant trend to gradually replace or at least decrease the amount of antibiotics, preservatives and material of animal-origin in vaccines, due to existing and potential safety concerns from contamination by adventitious agents or allergic reactions. For example, antibiotics have an increasing disuse in vaccines, mainly due to allergenic properties of some antibiotics in sensitive persons (anaphylaxis or local skin reactions). However, compared with others (*e.g.*, cephalosporins or sulfa drugs), the most commonly found antibiotics in vaccines are not known to cause severe allergic reactions (*e.g.* neomycin or polymyxin B), (Georgitis and Fasano, 2001). In addition, the antibiotic trace amount within vaccines is considered not significant, like the neomycin content in vaccines which is normally below 50 μg or lower, posing antibiotic content a very unlikely threat to elicit an allergenic response. Another preservative, the thiomersal (an organomercury compound) has been extensively used as a vaccine preservative for more than 80 years, but it is probably one of the most polemic vaccine excipient (Dorea *et al*., 2013). In 1999 in the USA, the thiomersal started to be substituted as vaccine preservative, and by 2003 no thiomersal vaccine was available in the USA (Food and Drugs Administration, 2013). The removal of thiomersal-containing vaccines in high income countries has recently fueled a continuous debate about discontinuation of thiomersal use in vaccines for human use altogether, questioning the benefits of the thiomersal ban in low and middle-income countries (King *et al*., 2013).

The preservatives are not the only vaccine components that have been subjected to controversy. Historically, the use of biological material of animal-origin (including human-origin) has been widely present in vaccine production and formulation, but these components are under constant safety surveillance due to the potential contamination by adventitious agents. The fetal bovine serum (FBS) is one example of animal-origin material used for decades for the cell culture-based vaccine production as a growth additive. The FBS is the most widely used growth additive, but other animals are also used for serum production. Over decades, progressive layers of regulatory restrictions and quality control tests along the vaccine production process have been put in place to minimize the possible contamination of vaccines by adventitious agents. Moreover, serum production is strictly regulated internationally by the European Medicines Agency and the Food and Drug Administration (FDA), and only good manufacturing practices (GMP) grade-certified products tested to be negative for a panel of adventitious agents, can only be used for vaccine development and manufacturing. One of the main animal-origin material risks is the bovine spongiform encephalopathy (BSE) in bovine-origin products (Baker and Ridley, 1996), including FBS (European Agency for the Evaluation of Medicinal Products, 2009). So far, there are no reported cases of BSE in humans transmitted via vaccines, remaining thus just as theoretical risk.

Even though gelatin is considered as generally regarded as safe (GRAS) excipient, its animal-origin still rises concerns about its safety due to presence of adventitious agents or allergenic properties (Rottem and Shoenfield, 2004), but only extremely rare incidence of anaphylaxis (one per 2 million cases) or urticaria has been reported when gelatin was used as stabilizer (Offit and Jew, 2003). Gelatin is a key component for the lyophilization process of most LAV, so gelatin removal from LAV formulations will be difficult until a suitable viable substitute will be available, being the recombinant human gelatin a promising candidate (Liska *et al*., 2007). Another commonly used animal-origin vaccine stabilizer is the human serum albumin (HSA), that have been used widely in LAV and combinatory vaccines (Supplementary Table S3), and reported to be linked with enhanced stability of flavivirus vaccine (Wiggan *et al*., 2011). As an animal-origin product, HSA raises the same concerns as serum or gelatin, and even when HSA has never been linked with any disease transmission in vaccines, again the theoretical risk exists. The FDA requests that vaccines containing HSA have a package label concerning the risk of viral disease transmission or vCJD included. Therefore, there is an increasing trend to substitute the HSA by recombinant albumin of alternative non-animal sources or by mixture of amino acids. For example, the Recombumin® from Albumedix is the first recombinant albumin approved for drug and vaccine manufacturing (Albumedix, 2016). Such increased availability of GMP manufactured non-animal origin amino acids, sugars or recombinant proteins as alternatives for replacement of "classical" animal-origin materials (HSA or gelatin), is one of the most promising strategies for the maximal decrease of adventitious agent's contamination for vaccine production.

Besides the excipients safety concerns, there is also safety concern about the final propagation or production substrate for viral vaccines, were there is a clear tendency to select cell substrates widely regarded as safe. The current selection of substrates varies according to the vaccine type, corresponding also to the vaccine API complexity. For example, for LAV manufacture the primary human cells (WI-38, MRC-5) are mainly chosen, but recently the transformed Vero cell line has been progressively used also, while for IN vaccines the specific pathogen free chicken eggs (SPFCE) represent still the most used substrate. The SUB vaccines are the most substrate innovative vaccine type, where yeast and bacteria are also used with success (Supplementary Table S3). The innovation in vaccine substrates faces the reluctance from vaccine developers, due to complicated, long and costly new

regulations and recommendation for the acceptance of new vaccine substrate (World Health Organization, 2013).

4. Cold chain and thermostable vaccine innovation in industry

The vaccine thermostability is the maintenance of a determined minimal viral potency in a specific thermal range during its shelf-life, and it depends on the vaccine type, API and route of delivery (Kumru *et al.*, 2014). Several private, governmental and academic entities have recognized the current challenge that thermostability poses in the vaccination programs throughout the world. As a major call for this urgent problem, "vaccines that do not require refrigeration" is currently one of the 16 grand challenges in global health put forth by the Bill and Melinda Gates Foundation originally in 2003 (Global Grand Challenges, 2016). The maintenance of appropriate low temperatures for storage and transport of vaccines is paramount in the vaccine distribution network and is called cold chain. Historically, the cold chain maintenance has been focused on avoiding elevated temperatures as source of vaccine instability. Potency loss can be attributed to storage, distribution, vaccine preparation and to administration time gaps. But nowadays, it is the inadvertent exposure to low temperatures of several freeze-sensitive vaccines that represent a serious threat to the thermostability of IN vaccines, especially with IN vaccines containing aluminum adjuvant, where freeze-stable formulations are under development (Braun *et al.*, 2009a). For example, it is revealing that the 75%–100% of IN vaccine shipments have been exposed to freezing temperatures at least once (Matthias *et al.*, 2007), emphasizing the importance of this problem. Failures in the cold chain have been contributed to local outbreaks and disease resurgence in the developing world. For example, during vaccine distribution monitoring in Papua New Guinea, it was found that 100% of the vaccines were exposed to freezing temperatures, but rarely exposed to elevated temperatures (Wirkas *et al.*, 2007). Theoretically, the maintenance of cold chain is possible in every country, but there are several reasons that hamper its implementation, particularly in low income countries, such as: outdated or improper refrigeration equipment; interruptions of energy supply due not reliable source or infrastructure; poor compliance with cold chain procedures; inadequate monitoring; poor understanding and/or training (Kristensen *et al.*, 2011). Among the most important advantages of vaccines with improved thermostability to high and freezing temperatures, are health and economic aspects such as: the expansion of immunization coverage; minimizing the cost increase, and patient's access to fully potent vaccines; reduced vaccine wastage and turnover by increasing vaccine shelf life (Chen and Kristensen, 2009). On one hand, despite these evident benefits and urgent need for improved thermostability of vaccines, economic constrains, and investment recovery are still the major obstacles for thermostable vaccine development by vaccine manufacturers. Logically, new vaccine stability formulations with innovative improvements must occur within the early phases of vaccine development, but these innovations can impact the costs of vaccine reformulation, extra clinical trials and additional regulatory approvals. On the other hand, an optimization of the vaccine thermostability represents several advantages for vaccine manufacturer as well: higher bulk production efficiency, reducing costs in lowering recalls, temperature controlled storage and transportation. Unfortunately, despite the technological prowess of the thermostable vaccine improvements, the presence of thermostable vaccines in the market or at late clinical phases aimed to licensing is limited (Supplementary Table S3, S4). Therefore, for the pharmaceutical industry it is important to ensure investment recovery, to make thermostable vaccine development attractive. Notably, the customer acceptance and support from governmental regulatory authorities and policymakers, via incentives and guidelines, can make new thermostable vaccine formulations development more attractive to industry. On the other hand, manufacturer should provide an improved product with net potential market advantages over the existing ones with sufficient proves about safety and efficacy of new thermostable vaccines.

5. Stabilizers in licensed and experimental vaccines

As mentioned before, composition of viral vaccine formulation is highly variable depending on the vaccine type. Moreover, the information about licensed vaccine components is not always complete, and formulations with same API can be substantially different from one product to another. Using information from summary of product characteristics or package inserts, we collected data from about 71 licensed vaccines types (LAV, IN and SUB vaccines) and their formulations (Supplementary Table S3). Formulation data for 9 out of 71 vaccines were not available. We reason that the number of times a specific stabilizer is mentioned in different vaccine formulations provides approximate information about its spectrum of use. As expected, the collected data showed that stabilizers in current LAV formulations are mostly enriched by well-known GRAS protein excipients such as HSA and/or gelatin with 1+ amino acids, and 1+ sugar components (Supplementary Table S3). Most likely, these rich formulations are required due the high level of complexity and intrinsic instability of API in LAV and IN vaccines, requiring lyophilized formulation, were gelatin

plays a decisive stabilization role. For example, albumin and gelatin are present in 12% and 19% of LAV and IN vaccines, respectively, while in SUB formulations both stabilizers are absent (Supplementary Table S3).

Comparing the licensed vaccine with the experimental vaccine list consisting of 38 vaccines (Supplementary Table S4), the presence of albumin and gelatin in experimental vaccines is reduced by 50%. Within the available data about experimental vaccines, the albumin and gelatin are mentioned in 8% and 16%, respectively, only in LAV formulations (Supplementary Table S4). Interestingly, amino acids are used in both licensed and experimental vaccines with similar frequency, about 25-30% of all vaccine type's formulations containing at least one amino acid. Glutamate, arginine, histidine and alanine are the most commonly mentioned amino acids in both licensed and experimental vaccine formulations (Supplementary Table S3 and S4). Most of the formulations that include amino acids are LAV, while in IN and SUB formulations the amino acid presence is reduced or they are not present at all. Amino acid mixtures are used for increasing protein solubility and stability (Golovanov *et al*., 2004). There are several reasons for amino acid presence in the vaccine formulations, depending on the amino acid physicochemical characteristics. For example, the histidine has antioxidant and buffering properties, by scavenging $HO^{\cdot}$ radicals in solutions (Wade and Tucker 1998) and by controlling pH and stabilizing non-covalent interaction of solid state proteins (Chen *et al*., 2003; Tian *et al*., 2007). Another interesting amino acid is arginine, which is widely known for preventing protein aggregation by interacting with aromatic and charged protein residues (Shukla and Trout, 2010).

Another important stabilizer group within vaccine formulation are sugars (mono /di/polysaccharides). In fact, approximately one third of therapeutic proteins in the pharmaceutical industry are stabilized in sugar glasses, which emphasize their wide use as well as proven stabilizing and protective potential. The sucrose and sorbitol are the most commonly mentioned sugar stabilizers in our licensed vaccine list being present in 20% and 14%, respectively (Supplementary Table S3.). Interestingly, in the list of experimental vaccines the trehalose is the most commonly found stabilizer among sugars (32% of experimental vaccine formulations contained trehalose), while sucrose, mannitol and the polysaccharide inulin are used in experimental vaccines with similar frequency (10%–13% of formulations). There are several reports supporting the trehalose stabilizing and cryoprotectant properties (Cicerone and Douglas, 2012, Kaushik and Bhat, 2003), suggesting trehalose as the most promising candidate in future LAV vaccine formulations. Moreover, trehalose increasing inclusion in several experimental formulations (*i.e.* alternative delivery routes: microneedles, spray freeze-dried, liquid and spray dried powder) highlights its versatility and potential of its stabilizing properties. Besides trehalose, the inulin has been also reported as a promising cryo- and lyo- protectant, especially for virosomes (de Jonge *et al*., 2007).

6. Conclusions

Nowadays, the production of cheap, efficient and stable viral vaccines comprises of several strategic, technical and economical challenges to the pharmaceutical industry and health agencies worldwide. In addition, the presence of new or emerging infectious viral diseases, like the pandemic zoonotic influenza H1N1 appearance in 2009 (Fineberg, 2014), the Ebola virus emergency in Central Africa in 2014 (Elshabrawy *et al*., 2015), or the recent Zika virus outbreaks in Latin America (Jamil *et al*., 2016), together with the increasing chronic market competitivity, represent a continuous pressure for time and cost-efficient vaccine formulation and production. The development and market innovation of cost-effective thermostable vaccines can alleviate the vaccine demand, potentially being a game changer in low-resources settings for immunization coverage. Current development in vaccine formulation focuses on the importance of structural characterization of the vaccine API, and the need of a systematic formulation analysis to avoid strategic failure resulting in sub-optimal formulation (Morefield, 2011). Inadequate vaccine formulation can result in jeopardizing the safety, efficacy and/or stability of the vaccine, increasing subsequent costs related to recalls, distribution, storage or drop in sales. Current rational approach for vaccine formulation development should thus consist of: API biophysical characterization; adjuvants and stabilizers evaluation; interaction between API and other excipients; production quality control assessment; and chronologic monitoring of stability in real time and in accelerated conditions. The vaccine stabilizer selection shows a clear tendency for substitution of animal-origin material (FBS, gelatin, HSA) for recombinant material, and the progressive inclusion of a sugar component into experimental vaccines (*e.g.* trehalose or inulin) (Supplementary Table S4). When sugars are included in the formulation, upon dehydration, the proteins are embedded in a sugar glass matrix. Even in the sugar-glasses, protein degradation can occur by chemical (oxidation, hydrolysis, deamination) or physical (aggregation) changes. Unfortunately, the exact mechanism or factors that regulate this degradation process is not completely understood. But several hypotheses have been described in attempts to understand, characterize and quantify the protein degradation rates in sugar glass matrixes (*i.e.* water replacement or vitrification hypothesis), but the understanding of these is extremely complex (Cicerone and Soles, 2004). Overall, the correct selection of the viral stabilizer can have a profound impact on vaccine thermostability,

which is especially more important in LAV and IN vaccines than in SUB vaccines, where a more complex API structure generally requires a more complex stabilizer formulation.

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Supplementary information is available in the online version of the paper.

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Supplementary information

REVIEW

Viral vaccine stabilizers: status and trends

F. M. C. CARDOSO[1], D. PETROVAJOVÁ[1], T. HORŇÁKOVÁ[1,2]

[1]Research and Development Department, Imuna Pharm a.s., Jarková 17, 082 22 Šarišské Michaľany, Slovak Republic;
[2]Olomouc University Social Health Institute (OUSHI), Olomouc, Czech Republic

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Supplementary Table S3. Overview of currently licensed vaccines representatives (the vaccines in this list are categorized by vaccine type, depicting available data from supplementary package insert, product monograph or package insert)

	Product name	Company	Stabilizers	Pres/adm[1]	FPPS[2]	Storage	Shelf-life (months)	Thermostability	Ref
LIVE ATTENUATED									
Adenovirus	No trademark name	Barr Labs/ Teva Pharmaceuticals (USA)	monosodium glutamate, HSA[4], plasdone C, sucrose, D-mannose, D-fructose, dextrose	tablet (oral)	WI-38[5]	2-8°C (FrSen[6])	48	N/A[7]	SPC[8]
Influenza (seasonal)	FluMist	Med Immune (USA)	monosodium glutamate, hydrolyzed porcine gelatin, arginine, sucrose	liquid (nasal)	SPFCE[9]	2-8°C (FrSen)	4,5	12 h (25°C)	SPC
Influenza (pandemic)	NasoVac (H1N1)	SII[10] (India)	gelatin, sorbitol, L-alanine, L-histidine, tricine, L-arginine hydrochloride, LAH[11]	lyophilized (nasal)	SPFCE	2-8°C	9	N/A	SPC, Dhere *et al*., 2011
Measles	Attenuvax	Merck (USA)	hydrolyzed gelatin, HSA, sorbitol, sucrose	lyophilized (SC[12])	CEF[13]	2-8°C	24	8 months (20-25°C), 4 weeks (37°C), reconstituted: loss of 50% potency in 1 h (20-25°C), all potency in 1 h (37°C)	SPC
	M-Vac	SII (India)	hydrolyzed gelatin, LAH, tricine, alanine, arginine, histidine	lyophilized (SC/IM[14])	HDC[15]	2-8°C	24	N/A	SPC

	Product name	Company	Stabilizers	Pres/adm[1]	FPPS[2]	Storage	Shelf-life (months)	Thermostability	Ref
	Measles Vaccine	Biofarma (Indonesia)	gelatin, L-cysteine, D-sorbitol, lactose monohydrate	lyophilized (SC)	SPFCE	2-8°C	24	N/A	SPC
	Measles Vaccine	GPO[16] (Thailand)	HSA	lyophilized (SC/IM)	CEF	2-8°C	36	N/A	SPC
	Measles Vaccine	Institute of Immunology (Croatia)	LAH, gelatin, L-arginine HCL, L-alanine, sorbitol, maltose	lyophilized (SC)	HDC	2-8°C	24	N/A	SPC
	Rouvax	Sanofi Pasteur (France)	HSA, lactose	lyophilized (SC/IM)	CEF	2-8°C	36	N/A	SPC
	Rudivax	Sanofi Pasteur (France)	N/A	lyophilized (SC/IM)	MRC-5[17]	2-8°C	24	reconstituted: 8 h (2-8° C), 4 weeks (37°C)	SPC
Polio	TOPV[18] Polio Sabin	GSK[19] (Belgium)	L-arginine, polysorbate 80	liquid (oral)	MRC-5	frozen	18	6 months (2-8°C)	SPC
	TOPV: Polioral	Novartis (Italy)	LAH	liquid (oral)	Vero	frozen	24	N/A	SPC
Rotavirus	Rotarix	GSK (Belgium)	amino acids, dextran, sorbitol, sucrose	lyophilized (oral)	Vero	2-8°C	36	lyophilized: 7 days (20°C); reconstituted: 24 h (2-8°C)	SPC
	Rotateq	Merck (USA)	sucrose, polysorbate 80	lyophilized (oral)	Vero	2-8°C	24	48 h (9-25°C), 12 h (26-30°C)	SPC
Rubella	Rubella vaccine	SII (India)	N/A	lyophilized (SC)	MRC-5	2-8°C	24	N/A	SPC
	Meruvax II	Merck (USA)	sorbitol, sucrose, hydrolyzed gelatin, HSA, FBS[20]	lyophilized (SC)	WI-38	2-8°C	24	reconstituted: 6-8 h (20-25°C)	SPC
Smallpox	ACAM2000	Sanofi Pasteur (USA)	HSA, mannitol	lyophilized (PC[21])	Vero	frozen	84	lyophilized: 18 months (2°-8° C); reconstituted: 6-8 hours (20°-25°C), 1 month (2°-8°C)	SPC
Varicella	Varilix	GSK (Belgium)	amino acids, lactose, mannitol, sorbitol	lyophilized (SC)	MRC-5	2-8°C	24	reconstituted: 8 h (2-8°C), 1.5 h (25°C)	SPC
	Varivax	Merck (USA)	gelatin, monosodium L-glutamate, sucrose	lyophilized (SC)	MRC-5	frozen	24	lyophilized: 24 months (2-8°C), 4 months (15°C); reconstituted: 6 h (27°C)	SPC 50
Zoster	Zostavax	Merck (USA)	hydrolyzed porcine gelatin, monosodium L-glutamate, sucrose	lyophilized (SC)	MRC-5	frozen	18	lyophilized: 72 h (2-8°C)	SPC
INACTIVATED									
Hepatitis A	Vaqta	Merck (USA)	aluminium (adjuvant), sodium borate	liquid (IM)	MRC-5	2-8°C (FrSen)	36	lyophilized: 3 months (28°C); 12 months (37°C)	SPC

	Product name	Company	Stabilizers	Pres/adm[1]	FPPS[2]	Storage	Shelf-life (months)	Thermostability	Ref
Influenza (seasonal	Optaflu	Novartis (USA)	N/A	liquid (IM)	suM-DCK[22]	2-8°C (FrSen)	12	N/A	SPC
Influenza (seasonal)	Fluarix	GSK (Germany)	N/A	liquid (IM)	SPFCE	2-8°C (FrSen)	12	1 freeze/thaw cycle: no effect, 12w (20°C)	SPC Patois *et al.*, 2011
	Flulaval	ID Biomed/GSK (Canada)	N/A	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	Agriflu	Novartis (Canada)	potassium, sodium, magnessium and calcium salts	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	Fluzone	Sanofi Pasteur (USA)	gelatin, Triton X-100	liquid (IM)	SPFCE	2-8°C (FrSen)	N/A	N/A	SPC
	Afluria	CSL limited (Australia)	sodium, potassium and calcium salts	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	Fluvax	CSL limited (Australia)	sodium, potassium and calcium salts	liquid (IM/ deep SC)	SPFCE	2-8°C (FrSen)	N/A	N/A	SPC
Influenza (pandemic)	H5N1	Sanofi Pasteur (USA)	porcine gelatin, sucrose	liquid (IM)	SPFCE	2-8°C (FrSen)	N/A	N/A	SPC
	Fluvirin (H1N1)	Novartis (UK)	N/A	liquid (IM)	SPFCE	2-8°C (FrSen)	6	N/A	SPC
	H5N1	ID Biomed/GSK (Canada)	ASO3[23] (adjuvant)	liquid (IM)	SPFCE	2-8°C (FrSen)	24	N/A	SPC
	2009 H1N1	CSL limited (Australia)	N/A	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	2009 H1N1	MedImmune/ AstraZeneca (USA)	sucrose, monosodium glutamate, hydrolyzed porcine gelatin, arginine	liquid (nasal)	SPFCE	2-8°C (FrSen)	4,5	N/A	SPC
	Focetria (2009 H1N1)	Novartis (USA)	MF59[24] (adjuvant)	liquid (IM)	SPFCE	2-8°C (FrSen)	N/A	N/A	SPC
	2009 H1N1	Sanofi Pasteur (USA)	gelatin	liquid (IM)	SPFCE	2-8°C (FrSen)	18	N/A	SPC
	Humenza (H1N1)	Sanofi Pasteur (France)	AF03[25](adjuvant)	liquid (IM)	SPFCE	2-8°C (FrSen)	6	N/A	SPC
	Anflu (H5N1)	Sinovac (China)	$Al(OH)_3$ (adjuvant)	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	Arepanrix (H1N1)	GSK (Belgium)	ASO3 (adjuvant)	liquid (IM)	SPFCE	2-8°C (FrSen)	6	50°C, hemagglutinin stable for 1 h	SPC Health Canada, 2010
	Green Flu-S (H1N1)	Green Cross (Korea)	N/A	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	Pandemrix	GSK (Germany)	AS03 (adjuvant)	liquid (IM)	SPFCE	2-8°C (FrSen)	24	N/A	SPC
	Panvax (H1N1)	CSL Limited (Australia)	polysorbate 20	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
	Panenza (H1N1)	Sanofi Pasteur (France)	polysorbate 20	liquid (IM)	SPFCE	2-8°C (FrSen)	12	N/A	SPC
Influenza (pandemic)	Celvapan (H1N1)	Baxter (Austria)	polysorbate 80	liquid (IM)	Vero	2-8°C (FrSen)	12	N/A	SPC
Japanese encephalitis	Ixiaro	NOVARTIS/ Intercell (UK)	$Al(OH)_3$ (adjuvant), protamine sulfate	liquid (IM)	Vero	2-8°C (FrSen)	18	N/A	SPC
	JE-VAX	BIKEN (Japan)	gelatin	lyophilized (SC)	mice brains	2-8°C (FrSen)	18	lyophilized: 28 weeks (22°C), 1 month (37°C), reconstituted: 8 h (2-8°C)	SPC, Takaku *et al.*, 1968

	Product name	Company	Stabilizers	Pres/adm[1]	FPPS[2]	Storage	Shelf-life (months)	Thermostability	Ref
Polio	Imovax	Sanofi Pasteur (France)	HSA	lyophilized (IM)	MRC-5	2-8°C (FrSen)	36	N/A	SPC
	IPOL	Sanofi Pasteur (France)	2-4 phenoxy ethanol, formaldehyde	lyophilized (IM/ SC)	mVero[26]	2-8°C (FrSen)	24	N/A	SPC
	Poliorix	GSK (Belgium)	aminoacids, 2-phenoxyethanol, formaldehyde, polysorbate 80	liquid (IM)	Vero	2-8°C (FrSen)	36	N/A	SPC
Rabies	Havrix	GSK (Belgium)	$Al(OH)_3$ (adjuvant), amino acids, polysorbate 20	liquid (IM)	MRC-5	2-8°C (FrSen)	36	lyophilized: 3 weeks (37° C)	SPC
	Imovax	Sanofi Pasteur (France)	HSA	lyophilized (IM)	MRC-5	2-8°C	N/A	N/A	SPC
	RabAvert	Novartis (Germany)	gelatin, HSA, glutamate, sodium EDTA	lyophilized (IM)	CEF	2-8°C	36	lyophilized: 3 months (37°C)	SPC, Barth *et al.*, 1983
	Rabipur	Novartis (Germany)	potassium-L-glutamate, polygeline, sucrose	lyophilized (IM)	CEF	2-8°C	48	N/A	SPC
	Verorab	Sanofi Pasteur (France)	maltose, HSA	lyophilized (IM/ID)	Vero	2-8°C	36	N/A	SPC
Yellow fever	YF-Vax	Sanofi Pasteur (USA)	gelatin, sorbitol	lyophilized (SC)	ALVFCE[27]	2-8°C (FrSen)	12	lyophilized: 14 days (37°C), 4 days (47°C); reconstituted: 1 h (25°C)	SPC
	Stamaril	Sanofi Pasteur (France)	lactose, sorbitol, L-histidine HCL, L-alanine	lyophilized (IM or SC)	ALVFCE	2-8°C (FrSen)	36	reconstituted: 6 h (2-8°C)	SPC
SUBUNIT/ RECOMBINANT									
Hepatitis B	Engerix B	GSK (Belgium)	$Al(OH)_3$ (adjuvant), polysorbate 20	liquid (IM)	yeast (HBsAg[28])	2-8°C (FrSen)	36	lyophilized: 72 h (25°C)	SPC
	Recombivax HB	Merck (USA)	$Al(OH)_3$ (Adj)	liquid (IM)	yeast	2-8°C (FrSen)	36	N/A	SPC
Influenza	FluBlock	Protein Sciences (USA)	Polysorbate 20	liquid (IM)	SF9[28] HA-VLPs[30]	2-8°C (FrSen)	4	N/A	SPC
	Celtura (H1N1)	Novartis (Germany)	MF59 (adjuvant)	liquid (IM)	MDCK	2-8°C (FrSen)	6	N/A	SPC
Human Papilloma Virus (Pr)	Cervarix	GSK (Belgium)	AS04[31] (adjuvant)	liquid (IM)	BTI-TN5B1-4[32], L1-VLPs	2-8°C (FrSen)	48	1 month (25°C), 7 days (37° C)	SPC, Le Tallec *et al.*, 2009
	Gardasil	Merck (USA)	aluminium hydroxyphosphate (adjuvant), L-histidine, polysorbate 80	liquid (IM)	yeast VLPs	2-8°C (FrSen)	36	130 months (25°C), 18 months (37°C), 3 months (42°C)	SPC, Shank-Retzlaff *et al.*, 2006
COMBINATORY									
DT/Poliovirus/Hepatitis B	Pediarix	GSK (Belgium)	aluminium salts (adjuvant)	liquid (IM)	yeast/Vero	2-8°C (FrSen)	N/A	72 h (8-25°C)	SPC

	Product name	Company	Stabilizers	Pres/adm[1]	FPPS[2]	Storage	Shelf-life (months)	Thermostability	Ref
DTP/inactivated Polio Hepatitis A and B	Kinrix	GSK (Belgium)	$Al(OH)_3$ (adjuvant)	liquid (IM)	mVero	2-8°C (FrSen)	36	N/A	SPC
	Twinrix	GSK (Belgium)	aluminium phosphate/hydroxide (adjuvant), amino acids, polysorbate 20	liquid (IM)	MRC-5/ yeasts	2-8°C (FrSen)	36	2 weeks (21°C), 1 week (37°C)	SPC Causer, 2005
Measles, Mumps and Rubella	MMR II	Merck (USA)	hydrolyzed gelatin, recombinant HSA, sorbitol, sucrose	lyophilized (SC)	CEF/ WI-38	frozen	24	lyophilized: 7 days (37°C), reconstituted: 8 h (2-5°C)	SPC
	Trimovax Merieux	Sanofi Pasteur (France)	HSA	lyophilized (SC/IM)	CEF/ SPFCE/ WI-38	2-8°C	24	N/A	SPC
	Tresivac	SII (India)	none reported	lyophilized (SC)	HDC/ CEF	2-8°C	24	N/A	SPC
	Abhayvac 3	Indian Immunologicals Ltd. (India)	sorbitol, gelatin, L-arginin, L-alanin, maltose, LAH	lyophilized (SC)	HDC/ CEF	2-8°C	24	N/A	SPC
Measles, Mumps, Rubella and Varicella	Priorix Tetra	GSK (Belgium)	lactose, amino acids, sorbitol, mannitol	lyophilized (SC)	CEF/ WI-38	2-8°C	18	N/A	SPC
	ProQuad	Merck (USA)	sucrose, sorbitol, hydrolyzed gelatin, monosodium L-glutamate, HSA	lyophilized (SC)	CEF/ WI-38/ MRC-5	frozen	18	lyophilized: 72 h (2-8°C)	SPC

[1]Pres (Adm) = presentation (administration route); [2]FPPS = final propagation or production substrate; [3]Ref = references; [4]HSA = human serum albumin; [5]WI-38 = human diploid cell line from lung tissue; [6]FrSen = freeze sensitive; [7]N/A = data not available; [8]SPC = supplementary product characteristics; [9]SPFCE = specific pathogen free chicken eggs; [10]SII = Serum Institute of India; [11]LAH = lactalbumin hydrolysate; [12]SC = subcutaneous; [13]CEF = chicken embryonic cells; [14]IM = intramuscular; [15]HDC = human diploid cells; [16]GPO = Government Pharmaceutical Organization; [17]MRC-5 = normal human fetal lung fibroblast; [18]TOPV = trivalent oral polio vaccine; [19]GSK = Glaxo SmithKline; [20]FBS = fetal bovine serum; [21]PC = percutaneous route (scarification); [22]susMDCK = Madin Darby canine kidney cells grown in suspension; [23]ASO3 = composed of squalene, DL-α-tocopherol and polysorbate; [24]MF59 = adjuvant composed of squalene; [25]AF03 = adjuvant composed of squalene, sorbitan oleate, polyoxyethylene cetostearyl ether, mannitol; [26]mVero = Vero cells grown in microcarriers; [27]ALVFCE = avian sarcoma leukosis virus free chicken embryos; [28]HBsAg = hepatitis B virus surface antigen; [29]SF9 = clonal isolate of *Spodoptera frugiperda* Sf21 insect cells; [30]VLPs = viral-like particles; [31]ASO4 = adjuvant composed of monophosphoryl lipid A adsorbed on to aluminum hydroxide salt; [32]BTI-TN5B1-4 = insect cell line from *Trichoplusia ni*.

Supplementary Table S4. Representative experimental viral vaccines

	Product name	Company	Pres/adm[1]	Stabilizers	Adjuvant	Thermostability	Ref
INACTIVATED							
Influenza	Nanopatch (microneedles/ Fluvax)	University of Queensland/ CLS limited (Australia)	coated microneedles (ID^2)	trehalose	no	6 months (23°C), 8 h (37°C)	Chen X *et al.*, 2011
	Inactivated influenza nano emulsion	Nanobio/Merck (USA)	liquid (IN^3)	$W_{80}5EC^4$	nanoemulsion	1 months (2-8°C), 1 months (25°C)	Hamouda *et al.*, 2010
	Influenza virosomes	University of Groningen (The Netherlands)	lyophilized (injected)	inulin	virosomes	12 weeks (20°C), 3 weeks (42°C)	de Jonge *et al.*, 2007
	Influenza virosomes	Pevion (Switzerland)	lyophilized	sucrose, (DC-chol, TC-chol, DOTAPDHAB)[5]	virosomes	>12 months (25°C), F/T[6] resistant	Kammer *et al.*, 2007

	Product name	Company	Pres/adm[1]	Stabilizers	Adjuvant	Thermostability	Ref
	Dry powder influenza vaccine	University of N. Carolina at Chapel Hill (USA)	SFD[7] (IN)	trehalose	no	>12 weeks (25°C), 2 weeks (37°C)	Garmise *et al.*, 2007
	Dry powder influenza vaccine	PowderJect (USA)	SFD[7] (ID)	trehalose, mannitol, dextran	no	>3 months (40°C)	Maa *et al.*, 2004
	Inactivated influenza vaccine	Stabilitech (UK)	lyophilized (SC)	N/A	no	>6 months (45°C)	Stabilitech, 2011
	Thermostable IM flu	Variation Biotechnologies (USA)	lyophilized (injected)	N/A	LPV[8]	6 months (40°C)	VBI vaccines, 2016
Pandemic	Whole inactivated H5N1	University of Groningen (The Netherlands)	lyophilized (Injected)	inulin	no	>1 year (25°C); 3 months (40°C)	Geeraedts *et al.*, 2010
	H5N1	Apogee Technology (USA)	injected	PCPP[8]	PCPP	30 h (40°C)	Andrianov *et al.*, 2011
VIRUS VECTORS							
Adenovirus	Adenovirus	Stabilitech(UK)	liq / lyo	N/A	N/A	liquid 6 months (2-8°C), lyo. 3 months (37°C)	Stabilitech, 2012
Influenza	ND1.1 (Adenovirus vector-HA-dsRNA) (H5)	Vaxart (USA)	dried capsules (oral)	not known	dsRNA (TLR3 antagonist)	1 month (25°C), 3 months (40°C)	Vaxart , 2016
	Recombinant Modified Vaccinia Ankara	Erasmus University (The Netherlands)	injected	not known	no	4 weeks (37°C)	Rimmelzwaan and Suttter, 2009
Japanese encephalitis	Vero cells derived JE vaccine	Kitasato Institute (Japan)	liquid (injected)	glycine, sorbitol	no	12 months (2-8°C), 12 months (28°C)	Toriniwa and Komiya, 2008
Yellow fever (En)	XRX-001 (inactivated YF vacine)	Xcellerex (USA)	liquid (injected)	proprietary stabilizers	$Al(OH)_3$	6 months (2-8°C), 8 weeks (25°C)	Monath *et al.*, 2010
LIVE ATTENUATED							
Dengue	ChimeriVax Tetravalent	Sanofi Pasteur (France)	lyophilized (injected)	N/A	no	1 month (25°C), 7 days (37° C)	Guy *et al.*, 2011
	DenVax	CDC, Inviragen/ Takeda (USA/ Japan)	lyophilized (SC/ID)	trehalose, rec HSA, F127	no	freeze/thaw, resistant, 11 weeks (2-8°C), 7 days (25°C), 8 h (37°C)	Wiggan *et al.*, 2011
Yellow fever	YF vaccine	Biomanguinhos (Brazil)	liquid (injected)	hydrolysed gelatin, sucrose, amino acids	no	2 weeks (37°C)	Freire *et al.*, 2005
Measles	Measles vaccine dry powder (MVDP)	University of Colorado, SII (USA/India)	dry powder (CAN-BD)[9]	myo-inositol, +/- sorbitol or mannitol	no	6 months (25°C), 1 week (37°C)	Rota, 2011
	Measles vaccine	Aridis Pharmaceuticals, SII (USA/India)	spray dried (pulmonary, injected)	trehalose, sucrose, divalent cations, L-arginine	no	8 weeks (37°C)	Burger *et al.*, 2008
	Measles vaccine	Stabilitech (UK)	lyophilized (SC)	N/A	no	resistant to 5 freeze/thaw cycles; 4 h (37°C), 6 days (37°C)	Stabilitech, 2012
	Measles vaccine	TransForm Pharmaceuticals/SII (USA/ India)	liquid	porcine gelatin, sucrose, trehalose, glycine, serine, tricine	no	<1 log loss 8 h (40°C)	Schlehuber *et al.*, 2011
	Measles Vaccine	Aridis Pharmaceuticals (USA)	spray dried	gelatin, HA, glycerol, trehalose, sucrose, L-arginine	no	>0.7 log loss at 2 weeks (37°C)	Ohtake *et al.*, 2009

	Product name	Company	Pres/adm[1]	Stabilizers	Adjuvant	Thermostability	Ref
	Measles Vaccine	University of Kansas (USA)	liquid	porcine gelatin, mannitol, myo-inositol, proline, malic acid	no	retention of 50% - 70% of infectivity for 24 hours (21° C).	Kissman *et al.*, 2008
	Measles Vaccine	University of Colorado (USA)/ SII	spray dried	gelatin, LAH, myo-inositol, mannitol, L-arginine, L-alanine, L-histidine, tricine	no	0.6 log loss in 7 days (37° C)	Burger *et al.*, 2008
Mumps	Mumps vaccine	Serum Research Institute (Iran)	lyophilized (SC)	hydrolyzed gelatin, trehalose, sodium glutamate	no	reconstituted (predicted): 155 h (4°C), 79 h (25°C), 21 h (37°C)	Jamil *et al.*, 2014
Polio	Trivalent OPV	Sapporo Medical University (Japan)	liquid (oral)	sorbitol	no	7 days (37°C)	Shiomi *et al.*, 2003
	Trivalent OPV	Institute Pasteur (France)	liquid (oral)	MgCl2 + D2O	no	3-7 days (37°C), 3 days (45°C)	Crainic *et al.*, 1996
Rotavirus	Rotavirus vaccine	Aridis Pharmaceuticals, Johns Hopkins (USA)	thin film (oral)	N/A	no	N/A	Johns Hopskins University, 2007
SUBUNIT/RECOMBINANT							
Influenza	dry powder Influenza vaccine	University of Groningen (The Netherlands)	lyophilized (injected)	trehalose, inulin	no	inulin: 6 months (25°C), Trehalose: 6 months (45°C)	Amorij *et al.*, 2007
	Dry powder Influenza vaccine	University of Groningen (The Netherlands)	pulmonary	inulin	no	3 years (20°C)	Saluja *et al.*, 2010
Hepatitis B	Shanvac-B	Shantha Biotechnics, PATH (India)	spray dried (IM)	trehalose, mannitol	$Al(OH)_3$	2 years (37°C)	Chen D *et al.*, 2010
	Shanvac-B	Shantha Biotechnics, PATH (India)	liquid (IM)	phosphate, histidine	$Al(OH)_3$	6 months (37°C), 6 months (45°C), 9 weeks (55°C)	Jezek *et al.*, 2009
	Shanvac-B	Shantha Biotechnics, PATH (India)	liquid (IM)	phosphate, histidine, propylene glycol	$Al(OH)_3$	freeze/thaw resistant, 12 months (37°C)	Braun *et al.*, 2009b
	HBsAg-NE	University of Michigan, Nanobio (USA)	liquid (IN)	$W_{80}5EC$	nanoemulsion	12 years (2-8°C), 6 months (20-25°C), 6 weeks (40°C)	Makidon *et al.*, 2008
Influenza	pfMBP-HA fusion protein	Nature Technology Corporation (USA)	liquid (ID in mice)	N/A	recombinant flagelin	40 months (75°C)	Luke *et al.*, 2011
	Microneedles coated with influenza virus	Georgia Institute of Technology (USA)	coated microneedles (ID mice)	trehalose	no	1 day (37°C)	Kim *et al.*, 2010
Rotavirus	Bacillus subtilis spores expressing VP6	Tufts University (USA)	lyophilized (IN or Oral)	none	cholera toxin	spore heat stable.	Lee *et al.*, 2010

[1]Pres (Adm) = presentation (administration route); [2]ID = intra dermal; [3]IN = intra nasal; [4]$W_{80}5EC$ = nanoscale emulsion (<800 nm), containing surfactants, refined soybean oil, ethanol; stabilizers: [5]DC-chol = 3_-[*N*-(*N*_,*N*_-dimethylaminoethane)-carbamoyl] cholesterol hydrochloride, TC-chol = cholesteryl *N*-(trimethylammonioethyl) carbamate chloride, DOTAP = propyl]-*N*,*N*,*N*-trimethylammonium chloride, DHAB = dimethyldihexadecylammonium bromide; [6]F/T = freeze-thaw cycle; [7]SFD = spray freeze dried; [7]LPV = lipid particle vaccine; [8]PCPP = poly[di(carboxylatophenoxy)phosphazene]; [9]CANBD = carbon dioxide-assisted nebulization with a bubble dryer.