



## TECHNOLOGY OVERVIEW

# Protecting aluminum-adsorbed vaccines from freeze damage

PATH has developed a low-cost and straightforward technology for protecting aluminum-adsorbed liquid vaccines from the irreversible damage that results from freezing. Many current vaccines of importance to global health contain an aluminum salt adjuvant or its equivalent (such as aluminum hydroxide, aluminum phosphate, or calcium phosphate) and would benefit from the technology.

### APPLICATION

The freeze-protection formulation technology can be easily applied to all relevant existing vaccines, including diphtheria, *Haemophilus influenzae* type b, hepatitis A, hepatitis B, human papillomavirus, influenza, Japanese encephalitis, meningococcal, pneumococcal, whole cell pertussis (wP), acellular pertussis (aP), poliovirus, rabies, rotavirus, and tetanus vaccines, as well as combinations of these vaccines.<sup>1</sup> It can also be applied to aluminum salt-adsorbed vaccines in the research and development pipeline, including candidates against dengue, human immunodeficiency virus, malaria, respiratory syncytial virus, typhoid, *Shigella*, tuberculosis, and new combinations of existing vaccines (such as hexavalent vaccine containing diphtheria, tetanus, pertussis or DTP; hepatitis B; *Haemophilus influenzae* type b; and inactivated poliovirus).<sup>2</sup>

PATH is available to assist vaccine producers and product development partnerships interested in applying the technology to improve the stability and effectiveness of aluminum-adsorbed liquid vaccines.

### NEED FOR PROTECTION

All vaccines lose potency over time, and the rate of loss is temperature-dependent, which is why a cold chain is necessary for vaccine storage and transport. For some vaccines, cold temperatures are even more damaging than heat. Especially vulnerable are liquid vaccines that contain aluminum salt adjuvants, which boost the immunogenicity and efficacy of the vaccine antigens but also increase the sensitivity of the vaccine product to freezing. Freeze-thawing results in aggregation and sedimentation of antigen-adjuvant particles, which is associated with a loss of vaccine potency. A handful of non-adsorbed vaccines are also freeze sensitive, including inactivated poliovirus vaccine and some inactivated influenza vaccines.

Studies of cold chain performance have shown that exposure of vaccines to freezing temperatures occurs frequently in developing and industrialized countries. In some countries, up to 100% of vaccine shipments were exposed to freezing temperatures at least once during distribution.<sup>3</sup> Very low ambient temperatures might also result in the freezing of vaccines during transport, leading to reduced vaccine efficacy.<sup>4</sup>

## COST ANALYSES

Exposure to freezing temperatures can result in either vaccine wastage (when vaccines are discarded due to suspected damage) or inadvertent administration of subpotent vaccine, which increases the risk that beneficiaries are not fully protected from disease.

The economic cost and consequences of vaccine wastage and/or the administration of subpotent vaccines are hard to estimate, but the purchase of freeze-sensitive vaccines represented over 50% of the US\$749 million spent by the United Nations Children's Fund (UNICEF) on all vaccines in 2010.<sup>5</sup> The procurement of the freeze-sensitive pentavalent vaccine alone cost US\$292 million and represented UNICEF's largest single expenditure toward reducing child mortality in 2010.<sup>6,7</sup>

Preventing freeze damage to vaccines could also benefit vaccine manufacturers. Many producers have experienced problems with accidental vaccine freezing during shipping, raising concerns about the resulting liability, expense of replacing shipments, and decreased effectiveness of freeze-sensitive vaccines that have been exposed to freezing temperatures.

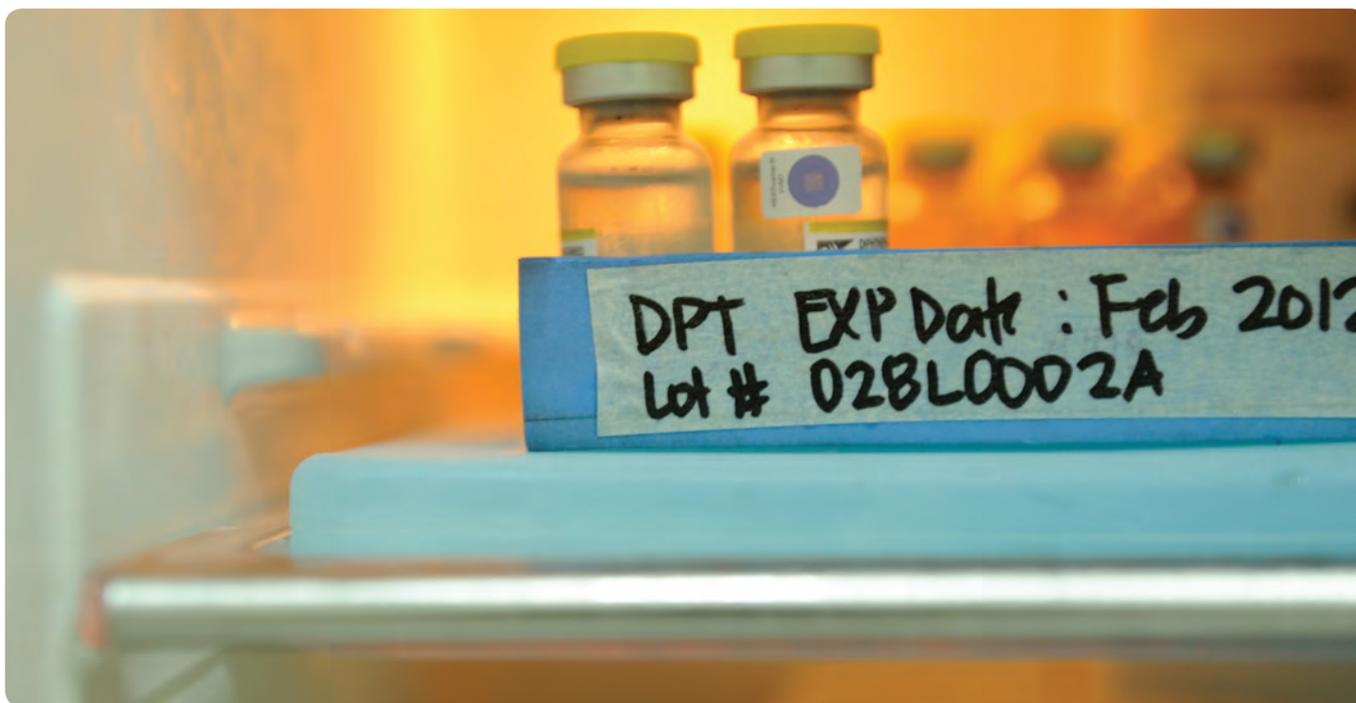
To understand the cost implications of different freeze-prevention strategies, PATH developed a model to simulate the transport and storage of 100 doses of pentavalent vaccine. In the assessment of four different

freeze-prevention scenarios, the model assumed the vaccine product was exposed to the identical temperature conditions recorded during a 2002 study conducted in two provinces in Indonesia.

Simulation results indicate that the freeze-protection formulation technology is the lowest-cost approach to ensuring vaccine potency following exposure to freezing temperatures. The next lowest-cost approach involves the use of carton-level indicators, an electronic refrigerator monitor, and a vaccine carrier with a phase change material liner. By comparison, the most expensive approach involves vial-level freeze indicators. Additional analyses are under way to understand the potential health economic impact of vaccine freezing under situations lacking any mechanism for freeze prevention.

## TECHNOLOGY SOLUTION

Freeze protection is achieved by adding a cryoprotection excipient—such as propylene glycol (PG), polyethylene glycol 300 (PEG 300), or glycerin—at low concentrations (5%–10% v/v) to the liquid vaccine formulation. The excipients protect the protein-adjuvant bond that would otherwise be disrupted by ice crystal formation during the freezing process. The excipients also help to maintain the antigen in a native form.



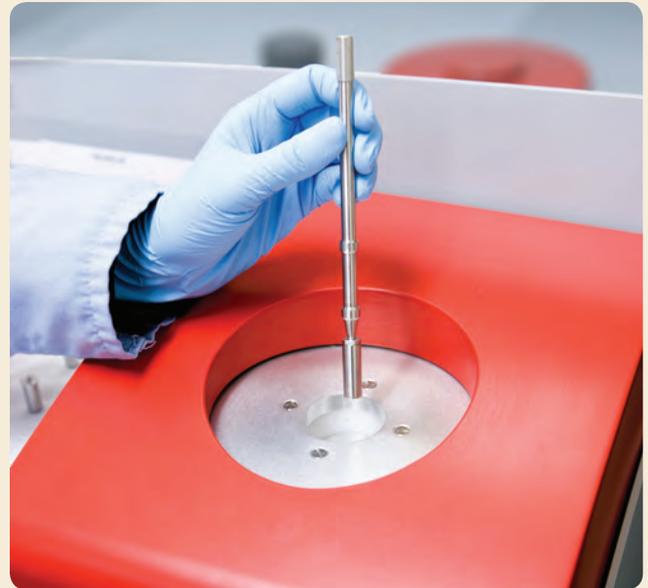
PATH/Tondo Manila

Exposure of vaccines to freezing temperatures occurs frequently and at various points in the vaccine supply chain.

## EVIDENCE BASE

For more than a decade, PATH has been researching the technical and commercial feasibility of improving vaccine stability. A key focus has been on freeze protection.<sup>8</sup> To assess the application of the freeze-protection technology to aluminum-adsorbed vaccines, PATH completed the following technical activities and studies.

- **Identification of cryoprotection excipients.** Three cryoprotective excipients have been identified: PG, glycerin, and PEG 300. Each stabilizer is miscible with water at any ratio; readily available at clinical grade; generally recognized as safe; and included in other parenteral, intramuscular, and subcutaneous drugs, including for pediatric use. Each also has a very low freezing point and costs less than US\$0.001 per vaccine dose.<sup>9</sup>
- **Development of *in vitro* assays and *in vivo* mouse models.** These assays and models helped to characterize freeze damage (e.g., structural changes and loss of immunogenicity) using commercially available adsorbed (i.e., adjuvanted with aluminum salts) hepatitis B and diphtheria, tetanus, and acellular pertussis (DTaP) vaccines.<sup>10,11</sup>
- **Baseline studies of the effects of freezing on commercially available hepatitis B vaccine.** Results indicated that freezing and freeze-induced damage of hepatitis B vaccine is a complex process that is dependent on formulation, temperature, and whether the vaccine is agitated during freezing. The studies also demonstrated that the adjuvant can be more sensitive to damage than the hepatitis B antigen itself.<sup>8</sup>
- **Evaluation of three excipients (PG, glycerin, and PEG 300) with hepatitis B vaccine.** Each of the three excipients (at 40%–50% v/v) protected a hepatitis B vaccine from structural damage at temperatures as low as -20°C (three freeze-thaw cycles) and prevented loss of immunogenicity in a mouse model.<sup>9</sup> PG appeared to have superior compatibility with hepatitis B vaccine and was cryoprotective at a broader range of temperatures and concentrations; 2.5%–10% v/v PG was cryoprotective.<sup>7</sup> More recent studies indicate that glycerin can protect against freeze damage at concentrations of 6%–8% v/v.<sup>12</sup>
- **Evaluation of three excipients (PG, glycerin, and PEG 300) with DTP vaccines.** The three excipients (tested at 30% v/v) prevented agglomeration of aluminum phosphate- or aluminum hydroxide-adjuvanted DTaP vaccines.<sup>7</sup> Studies conducted by a major vaccine manufacturer also indicated that 7.5%–10% PG protected against damage caused to a diphtheria, tetanus, and whole cell pertussis (DTwP) vaccine through three cycles of freeze-thawing to -20°C.<sup>13</sup>
- **Evaluation of PG with aP and wP.** PG was recently evaluated with DTaP and DTwP vaccines by another major vaccine producer. PG at 7.5% was able to protect both vaccines against three cycles of freezing at -20°C based on laboratory



PATH scientists use a calorimeter to determine the freezing point of vaccines.

analysis and standard potency tests in animals. The findings are as follows: immediately after three freeze-thaw cycles, the PG-formulated DTwP and DTaP (two batches each, 10 liters per batch) retained the original potency of wP and aP antigen components in the standard mouse potency tests. In contrast, the potency of wP and aP in the standard (non-freeze protected) vaccine fell below the acceptable level after freezing (the potencies of diphtheria and tetanus were not tested). The PG-formulated DTwP and DTaP vaccines, even after three cycles of freezing, had comparable stability to the standard vaccines (without freezing) after storage at 4°C for three months or at 25°C for 1 month.<sup>14</sup>

- **Evaluation of PG with a thermostable formulation of aluminum hydroxide-adjuvanted hepatitis B vaccine.** The cryoprotection excipient PG (7.5% v/v) and a thermostable formulation (phosphate and histidine buffers at pH 5.2) were found to be compatible, nontoxic, and effective at protecting against damage caused by freezing and storage at 37°C for 12 months.<sup>15</sup>
- **Preliminary evaluation of PG and glycerin with inactivated polio vaccine.** Glycerin and PG were each tested as excipients at a concentration of 10% v/v added to a trivalent inactivated polio vaccine stock formulation. Vaccine potency measured by ELISA was not adversely affected by excipients.<sup>14</sup>
- **Toxicity and safety assessments.** Single- and repeat-dose toxicology studies in rabbits with vaccines containing PG at 7.5% v/v suggest that the formulations are well tolerated with no acute toxicity.<sup>9,13</sup>

## INTELLECTUAL PROPERTY

There is no intellectual property barrier for manufacturers wishing to adopt the freeze-protection technology. It has been placed in the public domain.

## REGULATORY PATHWAY

The regulatory pathway for the freeze-protection technology is likely to be straightforward because it uses excipients that have established safety records. The technology does not require changes to production equipment, processes, packaging, or administration methods. In addition, integration of the freeze-protection technology with a new product will likely have a negligible impact on development or product costs. Relevant considerations prior to adoption include:

### For vaccines in development

- Laboratory and preclinical studies are required to demonstrate that the freeze-protection stabilizer is compatible with other vaccine components and to support the freeze-stability claim.
- Clinical studies should be conducted as part of the normal course of product development.

### For currently licensed products

- Laboratory and preclinical studies are needed to demonstrate the immunogenicity of the freeze-stable vaccine and its lack of interference with other concurrently administered vaccines.
- A bridging clinical study may also be necessary for demonstrating the non-inferiority of the freeze-stable vaccine to the current vaccine in use.



PATH/Oriental Mindoro

The freeze-protection technology can be applied to a number of existing and candidate liquid vaccines.

## CURRENT STATUS

PATH welcomes discussion and collaboration with vaccine developers and manufacturers interested in learning more about the technology, particularly for use with new aluminum-adsorbed vaccines of importance to developing countries.

## CONTACT DETAILS

For more information, including a technical dossier on the freeze-protection technology (updated in Q3 2012), please contact PATH's Vaccine and Pharmaceutical Technologies group at [vxpharmatech@path.org](mailto:vxpharmatech@path.org).

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