Effects of Freezing on Vaccine Potency

Literature Review

Hepatitis B

Diminsky D, Moav N, Gorecki M, Barenholz Y. Physical, chemical and immunological stability of CHO-derived hepatitis B surface antigen (HBsAg) particles. *Vaccine* 1999;18:3-17.

Recombinant hepatitis B surface antigen were stored at various conditions for 12-18 months in the naked form (HBsAg) or adsorbed to alum (HBV vaccine). HBsAg particles fully retained the original peptide composition when stored for 6 months, as a dispersion, at -20°C and 4°C; and as lyophilized powder at -20°C, 4°C, room temperature and 37°C. HBV immunogenicity was evaluated according to dose-dependent changes in antibody titers in immunized mice. The HBV vaccine ED50 for achieving seroconversion was 0.07 microg/ml/mouse, indicating that the vaccine is very immunogenic. However, freezing or freeze-drying of the HBV vaccine resulted in the total loss of vaccine immunogenicity (in spite of the good chemical stability). Yet full HBV vaccine immunological potency was retained for at least 2.5 years at 4°C. In conclusion, the HBV vaccine (but not naked HbsAg particles) lost its immunological potency upon freezing or freeze drying. The authors suggest that the HBV vaccine's physical, chemical and immunological characteristics are sufficiently stable at high temperatures to reduce the need for cold chain transportation.

McLean AA, Shaw R Jr. Hepatitis B vaccine. *Ann Intern Med* 1982;97:451.

This letter summarizes factors which pharmaceutical company investigators identified as contributing to lower than expected immunogenicity of inactivated hepatitis B vaccine in a CDC multi-center vaccine trial. Investigations centered on the shipment and storage of vaccine in part because laboratory data showed that freezing of hepatitis B vaccine causes formation of aggregates that reduced potency for humans. Assay of vaccine used in the trial indicated by aluminum sedimentation rates that freezing of vaccine occurred in three of the five centers. These three centers with evidence of vaccine freezing also had the lowest seroconversion rates. Freezing most probably occurred during shipment of the vaccine to the vaccine trial centers.

Klotz SA, Normand R, Silberman R. Hepatitis B vaccine in healthy hospital employees. *Infect Control* 1986;7:365-9.

A low rate of seroconversion to hepatitis B vaccine was reported and investigated by Veterans Administration Hospital in Shreveport, Louisiana. This occurred in healthy hospital employees from two separate institutions. Of 236 individuals evaluated in this study, only 53%, or 124 persons, developed protective levels of antibody to hepatitis B surface antigen following a complete vaccine series. In one hospital, 30% of the vaccine recipients developed antibody but not to a protective level. Vaccine was noted to have frozen in one hospital and accounted for some loss of antigenicity. This failure to respond to the vaccine has necessitated the use of booster injections of vaccine and continued antibody monitoring.

Edstam JS, Dulmaa N, Nymadawa P, Rinchin A, Khulan J, Kimball AM. Comparison of hepatitis B vaccine coverage and effectiveness among urban and rural Mongolian 2-year-olds. *Prev Med* 2002;34:207-14.

This evaluation of hepatitis B vaccine coverage and effectiveness compares the success of the immunization program between urban and nomadic rural populations. More than 95% of all subjects received hepatitis B vaccine, although rural subjects were less likely to complete the series than were urban subjects. Adequate vaccine response differed significantly as 94.2% of urban subjects versus only 70.2% of rural subjects had protective anti-HBs levels ($P < 0.001$). While the proportion of hepatitis B infection was lower than the historical prevalence, unexpectedly 40% of subjects in the rural population were found to be HBsAg positive. Explanation for why the vaccine response among rural subjects is less than that among urban subjects requires further study. Authors suggest freeze damage of hepatitis B vaccine in rural storage and transport as one explanation for the difference in vaccine response.

Pertussis

Milhomme P. Cold chain study: danger of freezing vaccines. *Can Commun Dis Rep* 1993;19:33-8.

This report details the danger of freezing vaccine as illustrated by epidemics of whooping cough among children who have received the pertussis vaccine. One factor contributing to the delivery of compromised vaccines with poor immunogenicity, reactogenicity, and efficacy is vaccine treatment during shipping and storage. A 1992 study in Canada by the Laboratory Centre for Disease Control used Freeze Watch™ indicators to document vaccine freezing during shipment in Canadian winters. In this 1993 study, Hib-TITER vaccine was evaluated to determine the time required for this vaccine to reach the freezing point when protected by special packaging. These results show that the internal temperature of the Styrofoam box decreases rapidly and that it is likely that vaccine shipments are compromised when shipped by surface and/or air freight during the winter and that alternative packaging is required for vaccine protection while not in heated environments during winter. This paper concludes with the importance of monitoring for vaccine inactivation with single-use or reusable freeze indicators and the shake test for adsorbed vaccines.

Boros CA, Hanlon M, Gold MS, Roberton DM. Storage at -3 degrees C for 24 hours alters the immunogenicity of pertussis vaccines. *Vaccine* 2001;19:3537-42.

The immunogenicity of pertussis antigens in an acellular and a whole-cell triple antigen vaccine used for childhood immunization was assessed in murine models after storage of vaccines below 0°C. Vaccines were stored at 2°-8°C (ideal), or at -3°C for 24 hours. Pre- and post-immunization IgG responses to pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) were measured using enzyme immunoassays (EIA). Responses to pertactin after receiving adversely stored DTPa were significantly reduced. A reduction in GMC to pertactin was also seen in response to adversely stored DTPw. Outbred mice receiving adversely stored DTPa had lower IgG antibody responses to FHA than those receiving correctly stored vaccine. Storage of pertussis vaccines below 0°C appears to alter the immunogenicity of PRN and FHA. Further study is required to determine the effects of such storage on vaccine protective efficacy.

Tetanus Toxoid

WHO. TT vaccine—safer out of the cold chain? *EPI Cold Chain Newsletter* 1990.

This article describes the likelihood that freeze-damage of tetanus toxoid (TT) vaccine, supplied by UNICEF and transported through the cold chain to Punjab, Pakistan, caused the unexpectedly low vaccine efficacy rate (as low as 27% in one district). This article suggests that in certain conditions TT may be safer out of the cold chain than within it.

Dietz V, Galazka A, van Loon F, Cochi S. Factors affecting the immunogenicity and potency of tetanus toxoid: implications for the elimination of neonatal and non-neonatal tetanus as public health problems. *Bull World Health Organ* 1997;75:81-93.

In 1989 WHO adopted the goal of eliminating NT as a public health problem worldwide. For this strategy to be effective, the TT used must be immunogenic. Potential factors that may affect TT immunogenicity need to be evaluated if NT is to be eliminated and if non-NT is to be controlled. Freezing TT has been shown to decrease its potency, but its impact on immunogenicity needs more evaluation.

Dietz V, Milstien JB, van Loon F, Cochi S, Bennett J. Performance and potency of tetanus toxoid: implications for eliminating neonatal tetanus. *Bull World Health Organ* 1996;74:619-28.

In certain countries the locally produced TT vaccine has been shown to be subpotent, while other countries have reported NT among infants born to vaccinated women. An extensive review of production and quality control procedures was carried out between 1993 and 1995 in 8 of 22 TT-producing countries that also report NT cases, with a more superficial assessment being carried out in the remaining 14 countries. Only 4 of the 22 countries have a functioning national control authority to monitor TT production and vaccine quality. A total of 80 TT lots from 21 manufacturers in 14 of the 22 NT-reporting countries were tested for potency. Of these, 15 lots from 8 manufacturers in 7 countries had potency values below WHO requirements. TT potency can also be compromised by improper vaccine handling. To eliminate neonatal tetanus worldwide requires assurance that all doses of TT meet WHO production and quality requirements and that the field effectiveness of TT is monitored through systematic NT case investigations and assessment of coverage.

Diphtheria-Pertussis-Tetanus (DPT)

Bass A. Trip notes: Visit to Al Hillah, 1985.

This trip report summarizes a retrospective investigation of eight NNT deaths where an estimated 80% of TT and DPT stocks at district and facility levels were found frozen or failed the shake test. General Establishment for Preventative and Environmental Health services Directorate of the Ministry of Health, Government of Iraq and UNICEF Baghdad.

Freezing and Thawing Experiment, Serum Institute of India, Ltd. 2002.

Investigators evaluated the effects of freeze-thaw cycles on tetanus, diphtheria, and pertussis toxoids by potency tests, toxicity tests, and physical parameters such as settling time. The potency of the tetanus component was evaluated on four batches of vaccine. After one freeze-thaw the end-point titration of the antibody induction method demonstrated an average of 85.5% of original potency (IU/mL) remained. After the second freeze-thaw cycle, potency was reduced to 38.5% of original potency, which remained near 40% after a third freeze-

thaw cycle. For the diphtheria component, potency was measured on three batches. After one freeze-thaw cycle, 94% of the original potency, as measured by antibody induction method, remained. After a second freeze-thaw cycle, 80% of the original potency remained. After the third freeze-thaw cycle, 44% of the original potency of the diphtheria component remained. A single batch of pertussis toxoid was evaluated by mouse potency assay (IU/dose). After a single freeze-thaw cycle, no potency was lost. After a second freeze-thaw cycle, 77% of potency remained. After three freeze cycles, pertussis toxoid potency was reduced to 45% of original potency. Settling time was non-compliant after a single freeze-thaw cycle for the TT, DT, Td, and DPT vaccine. All vaccines passed the undue toxicity tests for all three freeze cycles.

Brookman M. Safe Vaccine Storage Temperatures. WHO 1991. (A.9032)

Twenty-one brands of vaccine were tested to determine if they could be stored without freezing at temperatures equal to, or below their freezing point. The vaccines tested included TT, DT, DPT, and Hepatitis B. None of the vaccines tested exhibited any signs of freezing when stored for 16 hours at -1°C and -2°C, with and without tapping. Three of the vaccines, one DPT and two Hepatitis B, showed signs of starting to freeze after storage at -3°C for 16 hours and tapping. To avoid freezing damage to these vaccines it is best to avoid storing them in refrigerators that may go below 0°C.

The effects of freezing on the appearance, potency, and toxicity of adsorbed and unadsorbed DPT vaccines. *Weekly Epidemiological Record* 1980;55:385-92.

This paper summarizes evaluation by six laboratories of DPT vaccines, adsorbed and unadsorbed. Samples of several lots were stored at 4°C, stored at -5° to -15°C, and stored at -20° to -35°C. Potency was measured. A table summarizes the findings, with the conclusions stating that the shake test is useful for DPT and DT adsorbed vaccines, though not for unadsorbed vaccines. Potency data was not gathered for diphtheria toxoid; up to 25% reduction was seen in tetanus toxoid potency at temperatures of -30°C; pertussis saw up to 100% loss of potency at -30°C and 50% loss at -10°C. This article recommends gathering additional data.

Dimayuga R, Scheifele D, Bell A. Effects of freezing on DPT and DPT-IPV vaccines, adsorbed. *Can Commun Dis Rep* 1995;21:101-3.

This paper summarizes evaluation of whether DPT and DPT-IPV from Connaught Laboratories Ltd. are visually altered by freezing in response to concern that some adsorbed DPT products can be visually unchanged, despite loss of potency from freeze damage. Data from 80 vials tested under various conditions suggest that the shake test is ineffective in field conditions. Authors recommend that a temperature-monitoring device be included with vaccines during transport, either a min-max thermometer, temperature logger, or Freeze Watch™ indicators.

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REVIEW OF STABILITY DATA OF VACCINES AT TEMPERATURES BELOW 0°C

Component Tested	Vaccine Type	Brand	Scientific Freezing Point	Effect on Potency at Various Temperatures and Time Periods		
				0 to -10°C	<-10°C to -30°C	Repeated Freeze-Thaw
Hepatitis B[1]	plasma-derived and recombinant	N/A	-0.5°C	"As with other vaccines adsorbed on aluminum salts, freezing HB vaccine may cause a significant loss of potency."		
Hepatitis B[2]	recombinant	N/A	-0.5°C	"Vaccine batches should be stored at 2-8 C but not frozen. Freezing destroys the potency of the vaccine since it dissociates the antigen from the adjuvant alum interfering with the immunogenicity of the preparation."		
Hepatitis B[3]	recombinant	N/A	no data	"The HBV vaccine (but not naked HBsAg particles) completely loses its immunological potency upon freezing or freeze-drying."		
Hepatitis B[4]	recombinant	Recombivax (Merck)	no data	"Do not freeze since freezing destroys potency."		
Hepatitis B[5]	recombinant	Engerix B (GSK)	no data	24 hrs at -4°C still liquid, at -10°C some freezing occurs.	24 hrs at -20°C vaccine freezes and particles are agglomerated.	no data
Hepatitis B[6]	adsorbed HBV vaccine	N/A	no data	"As the adjuvant alum is added to the vaccine, freezing dissociates the antigen from the alum and interferes with the immunogenicity of the vaccine."		
Hepatitis B[7]	N/S	various	-1.0°C	Different HB vaccines froze only when exposed to temp between -5 and -11.8°C, although the scientific freezing point is -1.0°C.		
Tetanus Toxoid[8]	adsorbed DPT	N/A	no data	no data	no data	Immune response remained acceptably high in military recruits after 4 freeze-thaw cycles, although a decrease in mean immune response and lower proportion of high titres after 4 freeze cycles.
Tetanus Toxoid[8]	unadsorbed DPT	N/A	no data	no data	no data	Immune response remained acceptably high in military recruits after 4 freeze-thaw cycles.
Tetanus Toxoid[9]	unadsorbed DPT	3 labs, 3 vaccines	-5 to -15°C	At -5°C no significant decrease in potency, no change in physical appearance.	At -15°C and -30°C sample remained uniformly opalescent.	Coagulated and fibrous appearance after 6 cycles at -18°C.
Tetanus Toxoid[10]	adsorbed DPT	6 labs, 8 vaccines	-5 to -10°C	No decrease in potency in 5 vaccines tested. Change in appearance in 4 of 8 vaccines tested.	At -30°C, 25-29% reduction in potency in 2 of 5 vaccines tested and change in appearance in 6 of 8 vaccines tested. At -20°C, no reduction in potency in 3 of 5 vaccines tested.	no data
Tetanus Toxoid[9]	adsorbed DT	1 lab, 2 lots, 1 vaccine	-5°C	17% loss of potency at 12 hours with granular appearance.	At -30°C, 55% loss of potency at 12 hours with granular appearance.	>75% decline in potency after 6 cycles.
Tetanus Toxoid[11]	TT, Td, DT, DPT	Serum Institute of India, Ltd.	no data	no data	no data	Average reduction of potency of 4 formulas 15% after 1 cycle, to 61% after 2 cycles, and 60% after 3 cycles.
Diptheria[10]	adsorbed DPT	6 labs, 8 vaccines	-5 to -10°C	Change in appearance in 4 of 8 vaccines tested.	Change in appearance in 6 of 8 vaccines tested. No loss in potency of 2 vaccines tested at -30°C.	no data

Component Tested	Vaccine Type	Brand	Scientific Freezing Point	Effect on Potency at Various Temperatures and Time Periods		
				0 to -10°C	<-10°C to -30°C	Repeated Freeze-Thaw
Diphteria[9]	unadsorbed DPT	3 labs, 3 vaccines	-5° to -15°C	No differences in physical appearance in samples at -5°C.	No difference in potency for single vaccine frozen at -15°C or -30°C.	Coagulated and fibrous appearance after 6 cycles at -18°C.
DTaP, DTwP[6]				storage at < +2°C may cause antigens to fall from suspension and be difficult to resuspend		
Diphteria[9]	adsorbed DT	N/A	-5°C	no data	no data	>75% decline after 6 cycles.
Diptheria[11]	Td, DT, DPT	Serum Institute of India, Ltd.	no data	no data	no data	Average reduction of potency of 3 formulas 6% after 1 cycle, to 20% after 2 cycles, and 66% after 3 cycles.
Pertususis[10]	adsorbed DPT	6 labs, 8 vaccines	-5 to -10°C	17-50% loss in 2 of 5 vaccines tested. Change in appearance in 4 of 8 vaccines tested.	30-100% loss in potency in 3 of 5 vaccines tested. Change in appearance in 6 of 8 vaccines tested.	no data
Pertussis[12]	acellular and a whole-cell triple antigen vaccine	N/A	no data	Storage of pertussis vaccines at -3°C appears to alter the potency.	no data	no data
Pertussis[13]	adsorbed DPT and monovalent pertussis	N/A	no data	no data	after 15 days: < 50% potency remains.	no data
Pertussis[9]	unadsorbed DPT	3 labs, 3 vaccines	-5° to -15° C (range)	No differences in physical appearance in samples frozen at -5°C in all 3 vaccines.	In 1 of 3 vaccines, 34% loss in potency at -35°C but no physical change.	18% loss in potency after 6 cycles at -18°C single vaccine tested.
Pertussis[11]	DPT	Serum Institute of India, Ltd.	no data	no data	no data	Average reduction of potency 23% after 2 cycles, and 55% after 3 cycles.

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