RESEARCH

Use of Cool Water Packs To Prevent Freezing During Vaccine Transportation at the Country Level

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ABSTRACT: **Objectives:** To study the impact of the use of cool water packs (water packs refrigerated at 2 to 8 °C) on the cold life of vaccine transport boxes and the shelf life of the vaccines. **Methods:** Data loggers were used to measure the temperatures of vaccine shipments with cool water packs in laboratory studies and country evaluations. The temperature recordings were mathematically translated into reduction of vaccines shelf life, which are illustrated through degrees of color changes of Vaccine Vial Monitors. **Findings:** Laboratory studies at extreme ambient temperatures (43 °C) showed that, with the use of cool water packs, temperatures inside the cold box rise to around 20 °C within 48 h. When this exposure scenario was repeated four times, the impact of the temperature history on the different heat stability categories of vaccines varied between 2.4 and 36.0% shelf life loss. Oral polio vaccine was found to be the most affected vaccine. All other vaccines were affected with 2.4 to 10.4% life loss. Country assessments (real life situation with temperature variations between day and night) showed between 0.4% to 4.6% life loss when the boxes were exposed to ambient temperatures ranging from 11.7 to 39.8 °C over the 98 h 15 min test period. **Conclusions:** The use of cool water packs is found to be a legitimate and safe practice for vaccines other than oral polio vaccine, so that cool water packs can safely replace frozen icepacks without any serious consequences on the ability of vaccines to confer protection against disease.

KEYWORDS: Cool water packs, Freezing, Transportation, Vaccines, VVM, Nepal, Myanmar, Turkey, Zimbabwe.

Introduction

World Health Organization (WHO) guidelines recommend that liquid formulations of vaccines containing diphtheria, pertussis, tetanus, hepatitis B, *Haemophilus influenzae* type b and their combinations should not be frozen (1). Freezing of these vaccines provokes a loss of potency and, as a consequence, can result in compromised protective immunogenicity in recipients (2–5).

Freezing of vaccines occurs when vials are exposed to temperatures below 0 °C either during storage or transport depending upon a host of factors, including the duration that vaccine is exposed and whether the vaccine is agitated during that time period. Studies have shown exposure of vaccines to both subzero and freezing temperatures at all levels of the cold chain. Practices that put freeze-sensitive vaccines at risk are common not only in the developing world, but also in industrialized countries. Studies have shown freeze damage to vaccines in Australia (6, 7), Bolivia (8), Canada (9), Hungary (10), Indonesia (11), Malaysia (12), Papua New Guinea (13), the United Kingdom (14–17), and the United States (18).

The severity of the problem has been highlighted in a recent publication in which, of 14 shipments that were monitored, 12 experienced temperatures below 0 °C at one or more points in the cold chain in Indonesia (11). Ten of those were exposed to temperatures below 0 °C during district or sub-district transport in cold boxes.

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WHO recommends that, for the transport of freeze-sensitive vaccines, icepacks should be fully conditioned before being placed in the cold box with the vaccines (19). In order to do so, the icepacks should be kept at room temperature until the icepack temperature has reached 0 °C, that is, when the icepack contains a mixture of ice and water. The only way to check whether this is the case is to shake the icepack and verify whether the ice moves about slightly inside its container. Conditioning requires both space and, more importantly, time. An area of approximately 1 m^2 is needed to condition 25 icepacks, a number usually required for loading one large cold box. However, field observations show that programmes face a serious compliance problem in respect to icepack conditioning, indeed, this practice is generally found to be impractical and unrealistic because it requires more than 1 h at an ambient temperature of 20 °C. The practice of wrapping the freeze-sensitive vaccines to protect them from frozen icepacks and avoid freezing is found to be ineffective and no longer recommended by WHO (20).

The WHO Product Information Sheets define the *cold life* of a cold box and/or a vaccine carrier as the interval of time measured from the moment the coldest point in the load passes −3 °C until the temperature of the warmest point reaches 10 °C, at a given ambient temperature (usually 32 °C and 43 °C, respectively) (21). All the products listed in the "E04 cold boxes and vaccine carriers" section of the WHO Product Information Sheets have been tested in the laboratory and their cold life measured according to the above definition.

Recent discussions initiated by TechNet21 on icepack conditioning to prevent freezing have prompted the authors to take a close look at WHO recommendations on the transportation of vaccines (22). (TechNet21 e-Forum is a communication/information tool for generation of ideas on how to improve immunization services. WHO and UNICEF support TechNet21.) Between 2002 and 2004, a series of controlled laboratory studies and field tests were completed to assess the impact of using cool water packs (packs of water refrigerated between 2 and 8 °C) on the cold life of vaccine transportation boxes and on the shelf life of the vaccines.

Evaluations were conducted to verify the assumption that cool water packs can safely replace the use of icepacks for the transport of vaccines and, at the same time, prevent the freezing of vaccines.

This paper discusses the results of studies on the use of cool water packs conducted in controlled laboratory environments and in real life situations in Nepal, Myanmar, Turkey, and Zimbabwe.

Methods and Materials

Study Sites

Controlled environment laboratory and country level evaluations were performed to characterize the thermal performance of cold boxes loaded with cool water packs instead of frozen icepacks.

Laboratory evaluations at 43 and 32 °C were conducted at the Council of Scientific and Industrial Research (CSIR) of South Africa, at the BlowKings Ltd. manufacturing facilities in India, and in PT BioFarma, Bandung, Indonesia in 2002 (Tests at CSIR were conducted under WHO contract; other laboratory tests were financed/funded by BlowKings and PT BioFarma.) Country evaluations took place in Nepal, Myanmar, Turkey, and Zimbabwe between 2002 and 2004. Countries were recommended by WHO regional offices in an internal WHO meeting on the subject in December 2001.

Equipment and Supplies

Cold Boxes and Vaccine Carriers: The cold boxes/vaccine carriers used in the study included models RCW25/CF and RCW2/CF (manufactured by Dometic and tested at CSIR, South Africa), BK–VC 1.6CF and CB20–5U–CF (manufactured and tested by BlowKings, India) and the insulated international vaccine transport box (used in international shipments by PT BioFarma, tested at PT BioFarma, Indonesia). Insulated international vaccine transport boxes were also used in the Zimbabwe evaluation. The RCW25/CF was also used in tests in Nepal and Myanmar, while locally made vaccine carriers were used in the Turkey evaluation.

Empty icepacks were filled with tap water and placed in middle shelves of refrigerators (2 to 8 °C) over night. Cool water packs were then loaded into cold boxes/vaccine carriers to be exposed to ambient temperatures of 43 and 32 °C. All refrigerators used in cooling the water packs are special vaccine refrigera-

TABLE I
Methods Used in Controlled Environment Laboratory Tests[a]

Laboratory	Cold Box/Vaccine Carrier (Product Information Sheet Code)	Number and Type of Packs Used	Initial Temperature of the Packs	Ambient Temperature	Number of Thermocouples	VVMs Used
CSIR	RCW25/CF (E4/05-M)	24 × 0.6-L	2°C	43°C	3	YES
			8°C	43°C	3	YES
			2°C	32°C	3	YES
			8°C	32°C	3	YES
		No packs	n/a	43°C	3	YES
	RCW2/CF (E4/53-M)	2 × 0.3-L	2°C	43°C	3	YES
			8°C	43°C	3	YES
			2°C	32°C	3	YES
			8°C	32°C	3	YES
		No packs	n/a	43°C	3	YES
Blow Kings	BK-VC 1.6 CF (E4/83-M)	4 × 0.4-L	2°C	43°C	9	NO
	CB20-5U-CF (E4/76-M)	52 × 0.4-L	2°C	43°C	9	NO
PT BioFarma[b]	International transport box[c]	12 × 0.6-L	20°C	43°C	14	NO
		4 × 0.6-L	2°C			

[a] Dummy vials were used in all laboratory tests except the PT BioFarma test (see [c] below).
[b] PT BioFarma tests were conducted at 43°C ambient temperature with a DTP vaccine load and a mix of room temperature and 2°C cool water packs as part of the validation test of their packaging for international shipments, as required by the WHO vaccine prequalification scheme. The same test was repeated in three consecutive runs.
[c] Polyurethane box, outer dimensions 65 × 51 × 60 cm, inner dimensions 48 × 34 × 43 cm, weight 9.0 kg empty, loaded with combination of 12 × 0.6-L room temperature water packs and 4 × 0.6-L 2–8°C cool water packs and 2080 vials of 10-dose DTP vaccine.

tors listed in the WHO Product Information Sheet 2000 Edition (21). No electricity cuts and no temperature excursions were reported during the cooling process.

Temperature Monitoring Devices: During laboratory studies, temperatures were monitored at 5-min intervals for 48 h using a Grant Squirrel data logger with ±0.1 °C accuracy, using thermocouples at different locations of the load. For country evaluations, Tiny TTM® data loggers with ±0.3 °C accuracy (Gemini Data Loggers, UK) were programmed to record temperature at 3–5-min intervals for the whole transport period varying between 4 h (Turkey) and 98 h 15 min (Zimbabwe). Temperature monitoring devices were placed together with the vaccine load in the cold boxes. Different model data loggers with thermocouples inside of one of the icepacks were used to confirm temperature of cool water packs prior to loading.

In addition to these devices, vaccine vial monitors (VVMs) (Temptime, Morris Plains, NJ, USA) were shipped to CSIR and country study sites to be applied to the empty vials that constituted the dummy load used during the tests. All four types of VVMs were included (VVM2, VVM7, VVM14, VVM30). VVMs were not used in BlowKings and PT BioFarma tests.

Study Design

Laboratory Tests: Methods used in laboratory studies are shown in Table I.

The CSIR and BlowKings tests used dummy loads of vaccine vial, whereas, 2040 vials of diphtheria/tetanus/pertussis (DTP) vaccines were used in the PT BioFarma tests.

In the CSIR tests, three thermocouples were installed in vaccine carriers, one near the center in the bottom, one near the centre on the top, and one in the middle of the box. In the BlowKings tests, nine thermocouples were used, one in the each corner and one in the middle. In the PT BioFarma tests, a total of 14 ther-

mocouples were used. In all tests one additional thermocouple recorded the ambient temperature of the environmental chamber. Temperatures were recorded for at least 48 h.

Country Evaluations: Water packs cooled overnight between 2 to 8 °C were used to load the vaccine carriers and cold boxes. Tiny TTMs were programmed to measure, internal and ambient temperatures every 3–5 min. At least five routine in-country vaccine distributions were evaluated using real vaccines. In addition, four dummy vials labelled with four different types of VVMs were inserted in the load in all countries but Myanmar. A standard data entry form was used to collect data (refrigeration of the water pack and start and end time of each journey, VVM changes at the beginning and end of the each journey).

Estimation of the Remaining Shelf Life of Vaccines: The assumption is made that, if all factors affecting vaccine stability are controlled apart from exposure to heat, VVM reading can be used as a proxy indicator to determine the remaining shelf life of the vaccine. Indeed, all vaccines are assigned one of four types of VVMs on the basis of stability data generated by the manufacturer at the time of licensing or for prequalification purposes. These VVMs are assigned with a safety margin so that they reach their discard point before the exposure to heat has damaged the vaccine.

Recorded temperature data were used to calculate the amount of the VVM life which had expired.

The color change of a VVM over time is measured by its optical density (OD). (The optical density measured with the cyan filter of the X-Rite 404 GS (or GSX) Color Reflection Densitometer, or calculated from scanner measurements.) The rate constant, k, of the change varies with temperature according to the Arrhenius relationship:

$$k = A_o e^{-(E_a/RT)} \quad (1)$$

In this equation, A_o and E_a are experimentally determined constants specific to the reaction, and R is the universal gas constant. The activation energy, E_a, determines how the rate changes with temperature, T, which is expressed in degrees Kelvin. For a VVM30, the OD changes essentially linearly with time and reaches its end-point by 30 days at 37 °C, so the rate constant equals 1/30 per day at this temperature. The end-point is the stage where the difference between the OD of the reference ring and the OD of the active surface (center square) of the VVM reaches zero. The experimentally determined activation energy of 27.1 kcal/mole is used to determine the OD change for any other time–temperature combination or to plot an Arrhenius chart of the end-point as a function of temperature.

For details of VVM types and reaction rates, see Box 1. VVMs provide a visual guidance to the end users. In addition, precise VVM life loss was calculated as a percentage for study purposes.

Results

Laboratory Studies: Tests Conducted Under Controlled Environment Conditions

RCW25/CF Large Cold Box and RCW2/CF Small Vaccine Carrier: The results of all tests are summarized in Table II.

In all RCW2/CF tests, high temperatures, close to ambient, were recorded, and in all cases VVM2 reached their discard point within 48 h. At 43 °C for 48 h all VVM7 readings were close to end-point. On the contrary, although temperatures at 20 °C and above were recorded, in the large cold box all tests conducted with water packs resulted in acceptable VVM readings; thus one can extrapolate that the vaccine would not have been damaged by these exposures.

Based on the recorded temperatures, the remaining VVM life of the vaccines was calculated using the Arrhenius equation. Table III summarizes the findings.

BK-VC 1.6CF and CB20–5U–CF: One small vaccine carrier (BK-VC 1.6CF) and one large cold box (CB20-5U-CF) were tested with dummy vaccine loads and water packs refrigerated at 2 °C in an ambient temperature environment of 43 °C for 39 h and 116 h, respectively.

At the end of 5 days the highest temperature recorded in the large box was 20 °C while temperatures in the small vaccine carrier reached 30.4 °C at the end of 39 h. Because the temperature in small vaccine carrier was beyond 30.0 °C, the test was stopped at 39 h. The test with the large box was continued up to 116 h, the point when the box temperature reached 20 °C.

Box 1. VVM Types and Reaction Rates *(23)*

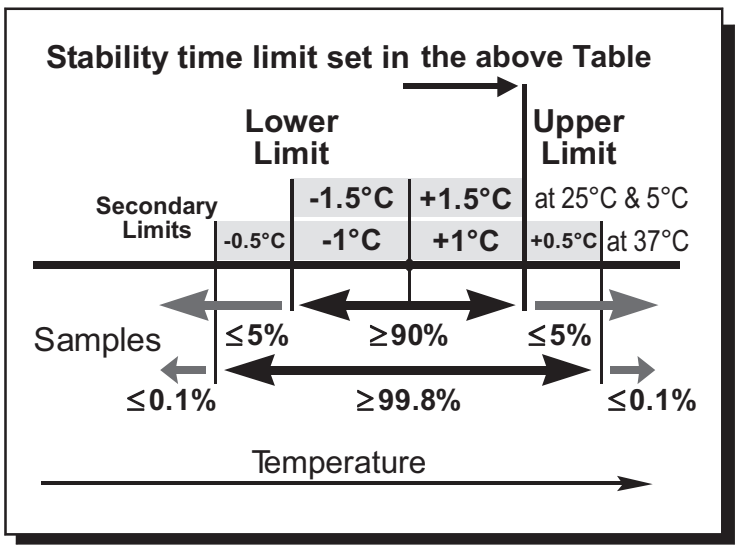
VVM Reaction Rates by Category of Heat Stability

Category: (Vaccines)	No. days to end point at 37 °C	No. days to end point at 25 °C	Time to end point at 5 °C
VVM30 HIGH STABILITY	30	193	>4 years
VVM14 MEDIUM STABILITY	14	90	>3 years
VVM7 MODERATE STABILITY	7	45	>2 years
VVM2 LEAST STABLE	2	NA*	225 days

**VVM (Arrhenius) reaction rates determined at two temperature points*

At 37 °C, relative humidity (RH) 33 ± 5% and RH 75 ± 5%, at least 90% of VVMs tested should reach the end-point at the maximum time in the range of 37 ±1 °C. Further, secondary limits are applied to restrict how far beyond the primary specification the time-temperature indicators (TTIs) are allowed to be. At least 99.8% of VVMs tested should reach the end-point at the maximum time in the range of 37 ± 1.5 °C. At the 5 °C and 25 °C specifications (ambient humidity in submerged foil/polythene pouch), at least 90% of VVMs tested should reach the end-point at the maximum time in the range of the specified temperature ±1.5 °C. A tolerance is allowed in the above tests for up to 5% of VVM samples tested to reach the end-point at a temperature above the upper limit and 5% at a temperature below the lower limit.

Stability Limit Criteria by Sample Group



Hypothetical used and remaining VVM lives of the vaccines were calculated as above. Table IV summarizes the findings.

PT BioFarma: Three consecutive runs with 2080 vials of DTP vaccine (conditioned at 5 °C) were conducted with a combination of 12 room temperature

TABLE II
Temperature Recordings of RCW25/CF and RCW2/CF During 48 Hours Exposure to 43°C and 32°C Ambient Temperatures at CSIR (in °C)

Box type	Ambient temperature	Initial water packs temperature	Temperature of the cold box/vaccine carrier[a]			VVM readings at the end of the test[b]			
			Average	Min	Max	VVM2	VVM7	VVM14	VVM30
Large RCW25/CF	43.0 ± 0.5	2 ± 0.5	11.5	2.5	20.0				
	43.0 ± 0.5	8 ± 0.5	16.3	8.2	23.6				
	43.0 ± 0.5	No packs	33.5	11.6	41.5				
	32.0 ± 0.5	2 ± 0.5	10.5	5.0	16.1				
	32.0 ± 0.5	8 ± 0.5	14.5	8.2	19.2				
Small RCW2/CF	43.0 ± 0.5	2 ± 0.5	34.1	3.1	42.7				
	43.0 ± 0.5	8 ± 0.5	35.1	9.1	42.2				
	43.0 ± 0.5	No packs	40.9	14.1	43.2				
	32.0 ± 0.5	2 ± 0.5	25.8	4.2	31.3				
	32.0±0.5	8 ± 0.5	27.4	10.8	31.7				

TABLE III
Remaining VVM Life After Storage in Large Cold Box and Small Vaccine Carrier Loaded with 8°C Water Packs, at an Ambient Temperature of 43°C for 48 Hours, CSIR Laboratory

VVM Type	VVM End-Point (Days)	Percentage of VVM Life Used	Remaining VVM Life in Days if Kept at 37°C
RCW25/CF large cold box			
VVM2	1.75	6.3	1.64
VVM7	6.125	1.8	6.02
VVM14	12.25	0.9	12.14
VVM30	26.25	0.4	26.14
RCW2/CF small vaccine carrier			
VVM2	1.75	138.4	Beyond the end-point
VVM7	6.125	39.6	3.70
VVM14	12.25	19.8	9.83
VVM30	26.25	9.2	23.83

TABLE IV
Remaining VVM Life After Storage in Large Cold Box and Small Vaccine Carrier Loaded with 2°C Water Packs, at an Ambient Temperature of 43°C, BlowKings

VVM Type	VVM End-Point (Days)	Percentage of VVM Life Used	Remaining VVM Life in Days if Kept at 37°C
CB20-5U-CF large cold box			
VVM2	1.75	0.7	1.74
VVM7	6.125	0.2	6.11
VVM14	12.25	0.1	12.24
VVM30	26.25	0.1	26.24
BK-VC 1.6CF small vaccine carrier			
VVM2	1.75	1.3	1.73
VVM7	6.125	0.4	6.10
VVM14	12.25	0.2	12.23
VVM30	26.25	0.1	26.23

(20 °C) water packs and four packs cooled at 2 °C, and the load was exposed to 43 °C ambient for 48 h. (These studies were conducted as part of the validation of shipping carton based on WHO requirements for international packaging and shipment. For this particular type of shipping carton, PT BioFarma had found that mix of room temperature and cool water packs was the best solution to prevent freezing and meeting WHO requirements at the same time.)

After 48 h, the highest temperature recorded inside the vaccine load was 25.3 °C. In this particular run, the median was calculated as 19.2 °C. Based on the temperature history, used and remaining VVM life of the vaccines was calculated using the Arrhenius equation. Results are displayed in Table V.

The highest impact was found in VVM2 (9.0% life lost) category vaccines. Despite this finding, if VVMs were attached, all readings would be within acceptable range for use.

Country Studies: Tests Conducted under Uncontrolled Environment Conditions

Country Evaluation—Nepal: A total of five shipments with cool water packs were monitored. Water packs were cooled overnight at the primary vaccine store cold room. In all shipments the RCW25/CF cold boxes were loaded with 20 water packs. Cold boxes were conditioned at room temperature. The temperature of all water packs was measured between 5 and 5.8 °C at the time of loading. All shipments contained 600 vials of 20 doses of DTP taken out from cold storage. In addition, dummy vials with four different types of VVMs were included in the box. Ambient and internal temperatures of the cold box were monitored with Tiny TTMs at 5-min intervals.

TABLE V
Remaining VVM Life After Storage for 48 Hours in International Transport Box Loaded with Combination of 2°C and Room Temperature Water Packs, at an Ambient Temperature of 43°C, PT BioFarma

VVM Type	VVM End-Point (Days)	Percentage of VVM Life Used	Remaining VVM Life in Days if Kept at 37°C
VVM2	1.75	9.0	1.59
VVM7	6.125	2.6	5.97
VVM14	12.25	1.3	12.09
VVM30	26.25	0.6	26.09

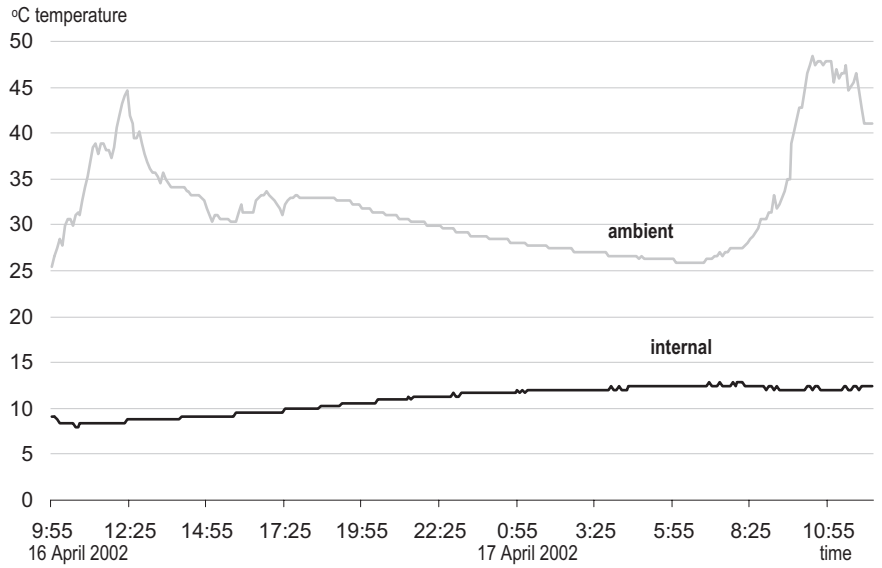


Figure 1

Temperature exposure during the longest journey, Nepal (584 km in 26 h 30 min).

The lowest temperature in cold boxes was recorded as 8.4 °C and the highest was 12.8 °C. During the journey ambient temperature varied between 25.9 and 48.4 °C.

In all shipments no visual changes in VVMs were observed. Therefore, used and remaining VVM life is calculated only for the longest journey.

Country Evaluation—Myanmar: Only one shipment was monitored in Myanmar, using five RCW25/CF cold boxes with 24 water packs cooled at 4 °C. Each cold box was equipped with two Tiny TTMs recording temperatures at an interval of 10 min (one at the bottom and one at the top of the load). Two additional Tiny TTMs were located outside the cold boxes to monitor ambient temperatures during the journey. Boxes 1–4 were loaded with 450 vials of 20-dose DTP vaccine, and box 5 contained 200 vials of 20-dose DTP and 280 vials of 20-dose tetanus toxoid (TT). No VVMs were used in the evaluation. Cold boxes were then loaded in an open truck. The journey of 867 km was completed in 72 h 42 min.

The box with the highest recorded temperature was used for the analysis. The lowest temperature in that box was recorded as 6.5 °C and the highest was 16 °C. During the journey, ambient temperature varied between 12 °C and 41.5 °C. The complete temperature history (ambient and highest internal recording) is displayed in Figure 2.

Based on the temperature history, used and remaining VVM life of the vaccines was calculated using the Arrhenius equation. Results are displayed in Table VII.

Country Evaluation—Turkey: The shipments monitored for the study were dispatches from the primary

TABLE VI
Used and Remaining VVM Life Based on Temperature History with RCW25/CF Loaded with 5°C Water Packs, Nepal

VVM Type	VVM End-Point (Days)	VVM Life Used (%)	Remaining VVM Life if Kept at 37°C (Days)
VVM2	1.75	0.7	1.74
VVM7	6.125	0.2	6.11
VVM14	12.25	0.1	12.24
VVM30	26.25	0.0	26.24

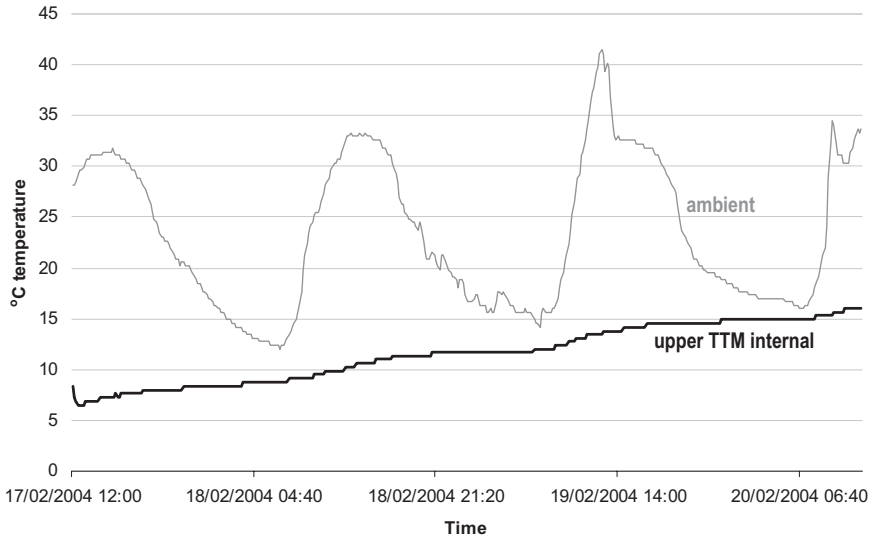


Figure 2

Temperature exposure during the journey, Myanmar (867 km in 72 h 42 min).

vaccine store to primary health care facilities located within a short distance. A total of 17 deliveries were monitored. In all deliveries locally made cold boxes (22 L) and small vaccine carriers (1.5 L) were used. The longest delivery time was 5 h. In all shipments water packs cooled at 5 to 6 °C were used. No temperature recordings were found to rise over 10 °C inside the cold boxes or vaccine carriers, while the average ambient temperature during deliveries was 20 °C. No change in the VVM could be observed.

Because of the short delivery time periods and the low temperatures recorded, no further analyses were conducted with the data collected.

Country Evaluation—Zimbabwe: A total of 11 deliveries were monitored with cool water packs and vaccine load. The temperature of the water packs was measured at 4 to 5 °C at the time of loading. All shipments used international insulated transport boxes loaded with 12 water packs of 0.4 L as well as 700 vials of 10-dose TT vaccine taken from the cold storage. Internal temperatures of the insulated boxes were monitored, at 3-min intervals, by a Tiny TTM placed at the top of the load. Distances varied between 80 and 440 km, and the longest journey was completed in 8 h 15 min. No change in the VVMs was observed.

In addition, an extreme case scenario was simulated: The insulated box with dummy load was placed in the booth of a private car which was parked in the sun during the day. The simulation lasted 98 h 15 min. The ambient temperatures varied between 11.7 and 39.8 °C, and temperatures inside the box varied between 7.7 and 21.3 °C, with an average of 16.1 °C.

Figure 3 shows temperature recordings during this simulation.

TABLE VII
Used and Remaining VVM Life Based on Temperature History with RCW25/CF with 4°C Cool Water Packs of 24, Myanmar

VVM Type	VVM End-Point (Days)	VVM Life Used (%)	Remaining VVM Life if Kept at 37°C (Days)
VVM2	1.75	2.9	1.70
VVM7	6.125	0.8	6.07
VVM14	12.25	0.4	12.20
VVM30	26.25	0.2	26.20

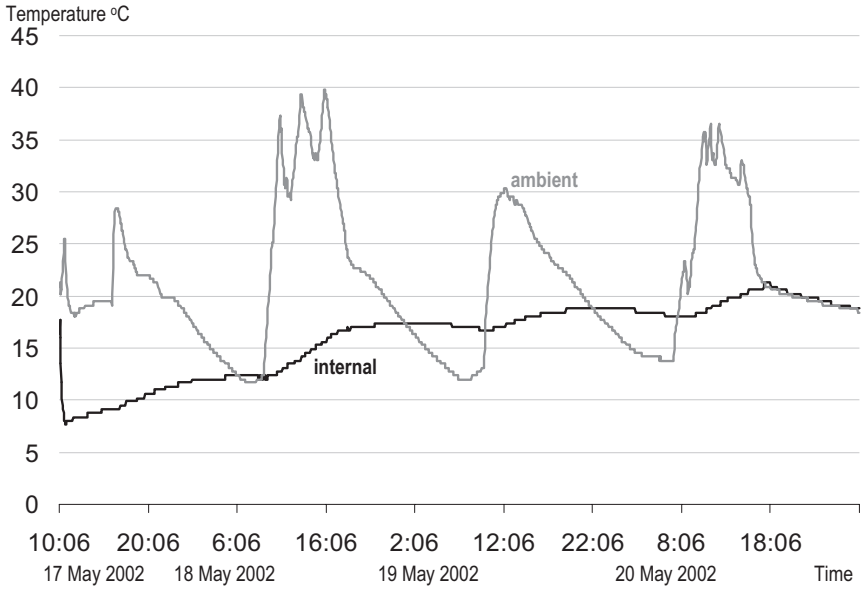


Figure 3

Temperature exposure during simulation, Zimbabwe (583 km in 98 hours 15 minutes).

Used and remaining life of VVM was calculated based on recorded temperature history using the Arrhenius equation.

Discussion

Recent publications show that vaccines are frequently exposed to negative temperatures at all levels of the distribution system, in industrialized countries as well as in developing countries (6–18). A recent systematic literature review highlights that accidental freezing is pervasive and occurs across all segments of the cold chain (24). Exposure to freezing temperatures occurs during transportation primarily because of the inappropriate use of deep-frozen icepacks. The conditioning of icepacks before loading is found to be time-consuming and impractical and is rarely being complied with. This has been verified during numerous field missions of WHO and UNICEF staff, and concerns were expressed by various country immunization managers (25).

These study results show that with large vaccine carriers at extreme ambient temperature of 43 °C, temperatures inside the cold box rose slightly above 20 °C, but did not substantially compromise the shelf lives of vaccines with VVM7, VVM14, and VVM30; all vaccines except oral polio vaccine (OPV) fall under these categories. This series of tests and field trials are naturally leading to the definition of a new concept: the *cool life* of a vaccine container where the vaccines are absolutely exempt from any risk of freezing.

The cool life is measured from the moment when the container is closed, until the temperature of the warmest point inside the vaccine storage compartment first

TABLE VIII
Used and Remaining VVM Life Based on Temperature History with Insulated Box with 5°C Cool Water Packs of 12, Simulation, Zimbabwe

VVM Type	VVM End-Point Days	Percentage of VVM Life Used	Remaining VVM Life in Days if Kept at 37°C
VVM2	1.75	4.6	1.67
VVM7	6.125	1.3	6.04
VVM14	12.25	0.7	12.17
VVM30	26.25	0.3	26.17

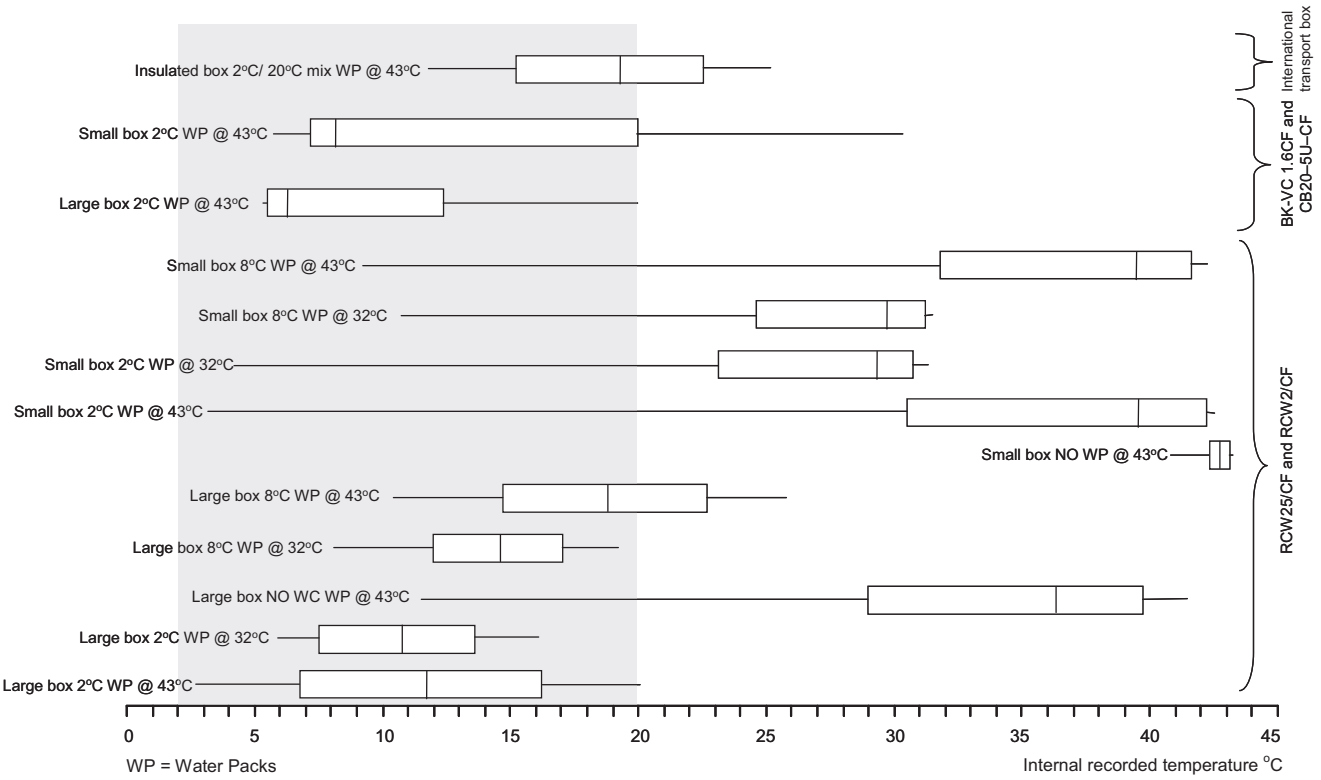


Figure 4

Box and whisker plot of recorded temperatures at all laboratory tests.

reaches 20 °C, at a constant ambient temperature of 43 °C.

Figure 4 displays minimum, maximum, median, 25th, and 75th percentiles of recorded temperatures at the laboratory tests.

The shaded area in the background of the box and whisker plot indicates the newly defined cool life. Although higher temperatures were recorded in the insulated box of PT BioFarma, VVM life loss was calculated between 0.6 to 2.7% in all vaccines but OPV. Naturally, small-volume boxes perform less optimal compared to large-volume boxes. The freezing risk is dependent also on the amount of insulation in the boxes. The boxes with better insulation are likely to be more risky if used with frozen ice packs (the long-life boxes having the highest risk). In general, large cold boxes are used for vaccine transportation from one store to another at the country level. Vaccine carriers are mainly used for bringing vaccines to the health centre as well as for outreach activities. Bringing vaccines to the health centre from the lowest level of storage facility is usually within hours and does not represent any risk to vaccines especially when used together with VVMs. Outreach transport may take extended periods of time (up to 48 h) and, most importantly, health workers need to have ice at the point of destination to keep reconstituted vaccines cold during the session. If cool water packs are used, there will not be "enough cold" for reconstituted vaccines. Therefore, use of cool water packs only applies to vaccine transport in between storage facilities and down to a fixed immunization point.

In a typical cold chain distribution system, vaccines travel from the primary vaccine store to intermediate level(s) down to the service level facilities. In most countries, there is only one intermediate level. Some large countries, however, can have up to three intermediate levels of vaccine stores (regional, provincial, and district) with vaccines making up to four trips to reach health facilities. The simulation below is drawn based on such a distribution system using temperature data generated at the laboratory tests above. The assumptions in the simulation are as follows:

TABLE IX
Repeated Temperature Exposure Impact on the VVM Life Simulation (Four Times of Transport at Ambient Temperature of 43°C for 48 Hours)

VVM Type (and End-Point Days)	VVM Life Used (%)	Remaining VVM Life if Kept at 37°C (Days)
1. RCW25/CF (Domestic)		
VVM2 (1.75)	25.2	1.3
VVM7 (6.125)	7.2	5.7
VVM14 (12.25)	3.6	11.8
VVM30 (26.25)	1.6	25.8
2. CB20-50-CF (Blow Kings)		
VVM2 (1.75)	2.8	1.7
VVM7 (6.125)	0.8	6.1
VVM14 (12.25)	0.4	12.2
VVM30 (26.25)	0.4	26.1
3. Insulated Box (PT BioFarma)		
VVM2 (1.75)	36.0	1.1
VVM7 (6.125)	10.4	5.5
VVM14 (12.25)	5.2	11.6
VVM30 (26.25)	2.4	25.6

- Vaccines are transported four times (primary to intermediate 1, intermediate 1 to intermediate 2, intermediate 2 to intermediate 3, and intermediate 3 to health centre)
- Only cold water packs are used
- Ambient temperature is constant 43 °C day and night
- Each and every transport takes 48 h

Repeated temperature exposure is applied to used and remaining VVM life calculation through the Arrhenius equation. Results are shown in Table IX.

In spite of the low impact calculated when the Blow-Kings cold box is used, the VVM2 (OPV) loses 25% of its life with RCW25/CF and 36% of its life with the insulated box. It is therefore obvious that OPV should not be transported with cool water packs. VVMs are classified according to vaccine stability (see Box 1). VVM category to vaccines is assigned by WHO experts based on the stability data of the vaccine examined. It is possible that the same type of vaccines produced by different manufacturers will be assigned a different type of VVM. However, in most cases, freeze sensitive vaccines such as DTP are assigned VVM14 and TT and HepB are assigned a VVM30.

Figure 5 shows the calculated highest impact on VVM readings in a scenario (number 3 in Table IX) in which the transport is repeated four times for 48 h at 43 °C

Figure 5

Temperature impact on VVM readings, insulated box.

ambient temperature, and an insulated box being used at all levels of vaccine distribution. The insulated box is a polyurethane box, outer dimensions 60 × 50 × 50 cm, inner dimensions 52 × 40 × 40 cm, weight 5.3 kg empty, loaded with combination of 12 × 0.6-L room temperature water packs and 4 × 0.6-L 2–8 °C cool water packs and 2040 vials of 20-dose DTP vaccine, total weight 43 kg.

As seen in Figure 5, it is not possible to visually detect any change in VVM14 and VVM 30. The change in the VVM7, although small, can be seen. Change can easily be seen with the VVM2, although the VVM has not reached the discard point (the vaccine remains usable) and lost only 36% of its shelf life.

Today WHO prequalified vaccines, with a few exceptions, comply with WHO and UNICEF requirements to affix VVM on vaccine vials (for vaccines that are available with VVM, refer to http://www.who.int/immunization_standards/vaccine_quality/pq_suppliers/en/index.html). The VVMs are designed to warn the user of the potential negative impact of an excess exposure to heat over time of the individual vaccine vials. Although developed as a heat-exposure indicator, VVM also contributes significantly to the reduction of vaccine freezing. VVM makes it possible to detect and avoid excessive heat exposure to vaccines when methods are employed to store and transport vaccines without ice and equipment that is a known source of freeze damage (26). Availability of VVMs are critical for introduction of cool water packs for in-country vaccine transportation systems to constitute a more flexible cold chain. The simulation is generated based on laboratory results, while there will be no case of continuous 43 °C ambient temperature for 48 h in real life. If real life examples are applied to a similar scenario, for example, the Zimbabwe simulation of 98 h 15 min transport is repeated 4 times with the same ambient temperature exposure, the highest loss would be found as 18% in VVM2 category vaccines.

The presence of ice below 0 °C is the main factor contributing to the freezing of vaccines during transport. The removal of ice eliminates the risk of vaccine freezing. The above results, however, demonstrate that the use of cool water packs is a safe practice for all vaccines except OPV. This clearly indicates that water packs can safely replace frozen icepacks without any damage to the vaccine potency or any major impact on vaccine shelf life. Successful implementation of this dual vaccine transport system (one for OPV and the other for remaining vaccines) has been observed in Moldova during an assessment (27).

One drawback to the use of cool water packs could be the refrigeration volume required to store water packs to cool for use when needed. Therefore, volume requirements for introduction of cool water packs should be carefully calculated.

Countries may consider conducting a temperature monitoring study in their vaccine cold chain before introducing cool water packs. Authors recommend the use of WHO study protocol developed for this particular purpose (28).

Based on the above findings, authors suggest the following steps to be followed for countries where the use of cool water packs in vaccine transportation is being considered (The process is not applicable to routes where vaccine transportation is done by using refrigerated vehicles).

1. Map vaccine distribution routes from primary store to all intermediate stores, and from intermediate stores down to service-level institutions.

2. Assess the maximum time required at each level of transportation.

3. Estimate the refrigeration capacity required to adequately cool water packs at each level and ensure that enough space is available (in case capacity is insufficient, locally produced refrigeration equipment can be used only for refrigerating the water packs).

4. Maintain a transport based on frozen icepacks for OPV and always separate OPV from all other vaccines during transportation. If a mixed load is to be carried, always use cool water packs.

5. Load cold boxes as recommended by the manufacturer, but using cool water packs rather than frozen icepacks.

6. Routinely check VVMs on arrival.

7. Introduce policy for all routes.

8. For maintaining a quality distribution, validate the temperature exposures inside the cold box using

electronic data loggers during the hottest and coldest time of the year. Assessments during the coldest time of the year are also important to decide whether "warm packs" are needed for extreme low ambient temperatures in cold climates (29).

In order to correct the problems identified during the cold chain study conducted in 2002 (11), Indonesia introduced the use of cool water packs for the transport of freeze-sensitive vaccines at the beginning of 2003 (30). Similarly, in 2004, Moldova adopted the use of cool water packs for all vaccines except OPV (27). Use of cool water packs was also been introduced for all vaccines in Zimbabwe following the temperature monitoring studies described in this article (31).

Limitations

Only several types of cold boxes and vaccine carriers were used in the study. The results obtained from the insulated container (polyurethane box) tests under a "worst case scenario" with repeated transportation legs at a constant 43 °C for a 48-h period provide good evidence to establish a policy for the use of cool water packs for in-country vaccine transportation. However, no generalization could be done for the other products listed in the WHO Product Information Sheets regarding whether they will develop similar results. In this regard, it is advisable that under the new WHO Performance, Quality and Safety (PQS) project (to replace the WHO Product Information Sheet system), a new verification protocol should be developed to document performance of cold boxes and vaccine carriers with cool water packs. The top point (20 °C) of the "cool life" could be used as a cut-off point in a verification protocol for the cold boxes. Without setting a standard for cold boxes, performance data could be displayed on the inside of the lid.

Post-submission note: Following the submission/acceptance of this article, on 8 December 2008 the PQS project has published new performance specifications and verification protocols for insulated containers (32–35). In these documents "cool life" is defined as "the empty container is stabilized at 43 °C and loaded with cool-packs which have been stabilized at 5 °C for a minimum of 24 hours. Cool life is measured from the moment when the container is closed, until the temperature of the warmest point inside the vaccine storage compartment first reaches 20 °C, at a constant ambient temperature of 43 °C." No standard has been set for the cool life, but the performance data is required to be permanently displayed inside the lid. For further details refer to http://www.who.int/immunization_standards/vaccine_quality/pqs_e04_insulated_containers/en/index.html.

Conclusions

Despite everything, we know that icepacks are not conditioned sufficiently and freeze-sensitive vaccines are exposed to freezing temperatures during their transport. It has been shown through several studies that the problem exists in countries regardless of their climatic situations (24). Although action is required to protect vaccines from freezing during transportation, and despite clear evidence of the use of cool water packs, it is unlikely that countries would change transportation policy that easily. Therefore, it is important that the use of cool water packs be translated into appropriate policies both at the global and local levels. Once cool water packs become a policy, use of conditioned icepacks would be a redundant approach mainly because of the risk of noncompliance and should not be promoted. Therefore, frozen and conditioned icepacks should be replaced with cool water packs for transportation of all vaccines at all levels of in-country distribution, except for OPV. The most direct clear benefit in doing so will be encouraging countries in the prevention of freezing vaccines.

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Conflict of Interest

None declared.

References

1. Milstien, J.; Kartoglu, U.; Zaffran, M. *Temperature sensitivity of vaccines.* World Health Organization: Geneva, 2006. WHO document WHO/IVB/06.10.

2. World Health Organization. The effects of freezing on the appearance, potency, and toxicity of adsorbed and unadsorbed DTP vaccines. *Weekly Epidemiological Record* **1980,** *55,* 385–392.

3. Klotz, S. A.; Normand, R.; Silberman, R. Hepatitis B vaccine in healthy hospital employees. *Infection Control* **1986,** *7,* 365–369.

4. Dimayuga, R.; Scheifele, D.; Bell, A. Effects of freezing on DTP and DTP-IPV vaccines, adsorbed. *Canada Communicable Disease Report* **1995,** *21-11,* 101–103.

5. 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. *Bulletin of the World Health Organization* **1997,** *75,* 81–93.

6. Gold, M. S.; Martin, L.; Nayda, C. L.; Kempe, A. E. Electronic temperature monitoring and feedback to correct adverse vaccine storage in general practice. *Medical Journal of Australia* **1999,** *171,* 83–84.

7. Burgess, M. A.; McIntyre, P. B. Vaccines and the cold chain: is it too hot . . . or too cold? *Medical Journal of Australia* **1999,** *171,* 82.

8. Nelson, C.; Froes, P.; Dyck, A. M.; Chavarria, J.; Boda, E.; Coca, A.; Crespo, G.; Lima, H. Monitoring temperatures in the vaccine cold chain in Bolivia. *Vaccine* **2007,** *25* (3), 433–437

9. Milhomme, P. Cold chain study: danger of freezing vaccines. *Canada Communicable Disease Report* **1993,** *19,* 33–38.

10. Lugosi, L.; Battersby, A. Transport and storage of vaccines in Hungary: the first cold chain monitor study in Europe. *Bulletin of the World Health Organization* **1990,** *68,* 431–439.

11. Nelson, C. M.; Wibisono, H.; Purwanto, H.; Manssur, I.; Moniaga, V.; Widjaya A. Hepatitis B vaccine freezing in the Indonesian cold chain: evidence and solutions. *Bulletin of the World Health Organization* **2004,** *82,* 99–105.

12. Hanjeet, K.; Lye, M. S.; Sinniah, M.; Schnur, A. Evaluation of cold chain monitoring in Kelantan, Malaysia. *Bulletin of the World Health Organization* **1996,** *74,* 391–397.

13. Wirkas, T.; Toikilik, S.; Miller, N.; Morgan, C.; Clements, C. J. A vaccine cold chain freezing study in PNG highlights technology needs for hot climate countries. *Vaccine* **2007,** *25* (4), 691–697.

14. Jeremijenko, A.; Kelly, H.; Sibthorpe, B.; Attewell, R. Improving vaccine storage in general practice refrigerators. *British Medical Journal* **1996,** *312,* 1651–1652.

15. Thakker, Y.; Woods, S. Storage of vaccines in the community: weak link in the cold chain? *British Medical Journal* **1992,** *304,* 756–758.

16. Wawryk, A.; Mavromatis, C.; Gold, M. Electronic monitoring of vaccine cold chain in a metropolitan area. *British Medical Journal* **1997,** *315,* 518.

17. Bishai, D. M.; Bhatt, S.; Miller, L. T.; Hayden, G. F. Vaccine storage practices in pediatric offices. *Pediatrics* **1992,** *89,* 193–196.

18. Bell, K. N.; Hogue, C. J.; Manning, C.; Kendal, A. P. Risk factors for improper vaccine storage and handling in private provider offices. *Pediatrics* **2001,** *107,* E100.

19. World Health Organization. *Guideline for establishing or improving primary and intermediate vaccine stores.* WHO/V&B/02.34; p 47.

20. World Health Organization. *Immunization in Practice.* WHO/IVB/04.06.

21. World Health Organization. Product Information Sheets 2000 Edition. WHO/V&B/00.13.

22. Protection of more cold sensitive vaccines. TechNet21 e-Forum. http://listes.ulaval.ca/listserv/archives/technet21e.html Post00391E (26 No-

vember 2001), Post00386E (23 October 2001), Post00400E (19 December 2001), Post00419E (30 January 2002), and Post00428E (19 February 2002).

23. World Health Organization. *PQS performance specification: Vaccine vial monitor. Specification reference WHO/PQS/E06/IN05.1).* Issued on 30 November 2006 (http://www.who.int/entity/immunization_standards/vaccine_quality/who_pqs_e06_in05_1.pdf).

24. Matthias, D. M.; Robertson, J.; Garrison, M. M.; Newland, S.; Nelson, C. Freezing temperatures in the vaccine cold chain: a systematic literature review. *Vaccine* **2007,** *25,* 3980–3986.

25. Kartoglu U. Chilled water packs study. TechNet21 e-Forum. http://listes.ulaval.ca/listserv/archives/technet21e.html Post00502E (20 September 2002).

26. World Health Organization. *Getting started with vaccine vial monitors.* WHO/V&B/02.35.

27. Babalioglu, N.; Kartoglu, U. *EVSM assessment: Chisinau primary vaccine store, Republic of Moldova. 6–10 December 2004* (unpublished EVSM external assessment report).

28. World Health Organization. *Study protocol for temperature monitoring in the vaccine cold chain.* WHO/IVB/05.01.

29. World Health Organization. *WHO-UNICEF Effective Vaccine Store Management initiative—Model quality plan, Module 2.* WHO/IVB/04.18.

30. Soepardi, J. Personal communication, Jakarta, March 2004.

31. Ganivet, S. Personal communication, Geneva, February 2005.

32. World Health Organization. *PQS performance specification: Vaccine cold box. Specification reference WHO/PQS/E04/CB01.1).* Issued on 8 December 2008 (http://www.who.int/entity/immunization_standards/vaccine_quality/who_pqs_e04_cb01_1.pdf).

33. World Health Organization. *PQS independent type-testing protocol: Vaccine cold box. Product verification protocol WHO/PQS/E04/CB01.VP.1).* Issued on 8 December 2008 (http://www.who.int/entity/immunization_standards/vaccine_quality/who_pqs_e04_cb01_vp1.pdf).

34. World Health Organization. *PQS performance specification: Vaccine carrier. Specification reference WHO/PQS/E04/VC01.1).* Issued on 8 December 2008 (http://www.who.int/entity/immunization_standards/vaccine_quality/who_pqs_e04_vc01_1.pdf).

35. World Health Organization. *PQS independent type-testing protocol: Vaccine carrier. Product verification protocol WHO/PQS/E04/VC01.VP.1).* Issued on 8 December 2008 (http://www.who.int/entity/immunization_standards/vaccine_quality/who_pqs_e04_vc01_vp1.pdf).