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Temperature Sensitivity of the Diphtheria Containing Vaccines

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1. Introduction

Immunization managers can improve the efficiency of immunization programmes through enhancing their knowledge of a vaccine's stability.

Vaccine management is basically all the actions related to handling of vaccines at the country level from the moment they arrive until the moment they are used. These include arrival and acceptance procedures, appropriate temperature monitoring, ensuring sufficient storage volume, maintaining standards of buildings, equipment and vehicles, effective stock management, vaccine delivery systems as well as effective use of policies such as the multi-dose vial policy (MDVP) and the use of vaccine vial monitors (VVM).

The World Health Organization (WHO) and UNICEF offer standard tools to effectively monitor management performance of vaccine stores and the vaccine management system in a country (World Health Organization, 2010).

Assessments conducted in various countries on effective vaccine management (EVM) indicate that maintaining equipment at the temperature range recommended by the WHO is not always observed (Milstien J et al., 2006). Moreover, in case of such violations, no proper follow-up actions are taken. Many countries still lack appropriate temperature monitoring tools for vaccine stores and refrigerators. Among the studies documenting temperature violations there are some that indicate that temperature violations may affect the diphtheria containing vaccines (Bishai et al., 1992; Burgess & McIntyre, 1999; Hanjeet et al., 1996; Lugosi & Battersby, 1990; Jeremijenko et al., 1996; Milhomme, 1993; Thakker & Woods, 1992; Wawryk et al., 1997; Wirkas et al., 2006). It has been observed that cold chain practices tend to rather prioritize protecting vaccine from heat damage, thus often creating the risk of exposure to freezing temperatures. As a result, inadvertent freezing of vaccines is a largely overlooked problem all over the world. In a recent systematic review, comparison of the occurrence of freezing temperatures during storage and transport were found to be a global problem occurring both in the resource-rich as well as the resource-limited settings (Matthias et al., 2007).

2. Stability of diphtheria containing vaccines

National regulatory authorities (NRA) establish the expiry dates for diphtheria toxoid vaccines through a licensing process applicable for each vaccine. In this licensing process,

the manufacturer provides data to support the claimed shelf life, although vaccine may still be efficacious beyond the claimed shelf life at 2-8°C.

2.1 Analysis of vaccine stability

2.1.1 Exposure to high temperatures

The stability of diphtheria toxoid is similar to that of any simple polypeptide, that is, unaffected by rising temperatures up to the point where secondary structure is lost: generally well above 50°C (Milstien et al., 2006). In monovalent or combination vaccines diphtheria toxoid is always adsorbed onto aluminium-based adjuvants. They are stable at elevated temperatures even at long periods of storage. On the contrary, diphtheria toxoid containing vaccines may change their appearance and lose potency when frozen due to freezing destroying the gel structure of the adjuvant. The shelf-life, at the temperature usually recommended by manufacturers (2-8°C), depends on the nature of the vaccine. Monovalent toxoid and combined diphtheria and tetanus toxoid vaccines have longer shelf life (usually three years) compared to DTP and DTP combination vaccines (18-24 months). In DTP and DTP combinations, the pertussis is the least stable component compared to both diphtheria and tetanus toxoids, therefore limiting the shelf-life.

Diphtheria toxoids exposed to 60°C are destroyed in three to five hours (Sporzynska, 1965).

2.1.2 Exposure to freezing temperatures

Adsorbed diphtheria vaccines, whether monovalent or combined, alter their physical appearance after freezing changes the structure and morphology of the aluminium adjuvant. Changes in pH and storage at higher temperatures have no influence on the structure of aluminium gel, but freezing causes extensive morphological changes that are visible under the phase-contrast microscope (PCM) and scanning electron microscope (SEM) (Aleksandrowicz et al., 1990; Kartoğlu et al., 2010a). The development of heavy conglomerates, floccules or other granular matter produces an increase in sedimentation rates (Shmelyova, 1976; World Health Organization, 1980; Aleksandrowicz et al., 1990; Kartoğlu et al, 2010a). The size of the granules seems to increase on repeated freezing and thawing. The time required to freeze diphtheria containing vaccines as well as all other freeze-sensitive vaccines depend on the number of doses in the vial (the greater the volume, the longer the time) and on the temperature exposed. Studies conducted by the WHO indicate that to freeze diphtheria containing vaccines around 110-130 minutes are required at -10°C, 25 to 45 minutes at -20°C, and 9 to 11 minutes at -70°C. Because of supercooling, the temperature in diphtheria containing vaccine vials falls to well below zero (-1.6°C to -2.6°C when the outside temperature is -4.2°C to -4.6°C) before reaching an unstable threshold. At the moment of solidification the temperature in the frozen vaccine rises to the scientific freezing point, which is about -0.5°C (World Health Organization, 1990). Phase change in freezing is also affected by the vibration where the vials are resting mainly by accelerating the friction among the molecules to trigger the crystallization.

The physical changes induced by freezing can be detected by the “shake test”, which is the only test that can detect freezing in all aluminium adjuvanted vaccines (World Health Organization, 1980; Kartoğlu et al., 2010a). A learning guide in Box 1 under section 3.3

explains how to do a shake test. WHO has also produced an educational video explaining how to conduct a shake test (Kartoğlu, 2010c).

The amount of antigen in a frozen non-homogeneous vaccine can vary greatly, and the administration of such a vaccine may be associated with a reduced immune response. Similarly, it may also be linked an increased incidence of local reactions due to an increased amount of aluminium adjuvant in the dose drawn for injection.

In diphtheria containing combination vaccines, reduction of the potency of different components evidently varies slightly depending on the composition of the vaccine. The tetanus toxoid component in two of five DTP vaccines stored for 12 hours at -30°C showed a decrease in potency of about 30%, while there was no such decrease in vaccines kept at -5°C to -10°C. However, the potency of the tetanus toxoid component in adsorbed DT vaccine was reduced after freezing at both -5°C and -30°C (World Health Organization, 1980). This difference is undoubtedly due to the aluminium adjuvant effect of the pertussis component in the DTP vaccines when the potency is tested by animal assay. The relevance of this observation to protective efficacy is not known. Since it would be unethical to conduct studies with known frozen vaccines, real efficacy data are difficult to get as each product has its own particular threshold for freeze damage. This also shows that there is a difference between exposure to freezing temperatures and actual freezing to destroy the potency. That is why the shake test is so important to decide whether vaccines are affected by freezing.

A study performed by Serum Institute of India Ltd. on their own DT, Td, and DTP vaccines using three freeze-thaw cycles gave the results presented in Fig 1 (Serum Institute of India, 2005).

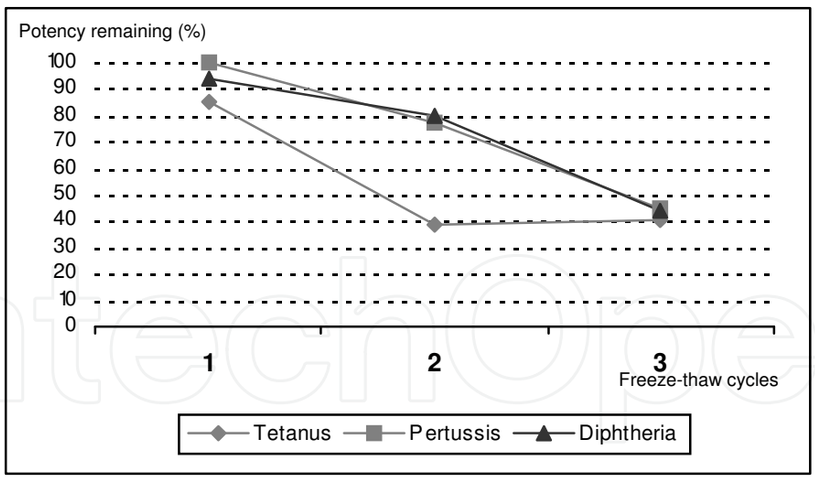


Fig. 1. Results of freeze-thaw cycles on potency of adsorbed DTP vaccine from Serum Institute of India.

3. Ensuring the optimal potency of vaccine

3.1 Temperature control requirements for diphtheria containing vaccines

To ensure the optimal potency of vaccines, careful attention is needed in vaccine handling practices at the country level. These include storage and transport of vaccines from the

primary vaccine store down to the end-user at the health facility, and further down at the outreach sites. The WHO recommended conditions for storing the diphtheria containing vaccines used in immunization programmes are shown in Table 1. This Table also indicates the maximum storage periods and temperatures in each case.

	Primary vaccine store	Intermediate vaccine store		Health centre	Health post
		Province	District		
Storage temperature	+2°C to +8°C	+2°C to +8°C	+2°C to +8°C	+2°C to +8°C	+2°C to +8°C
Maximum storage period	6-12 months	3 months	1-3 month	1 month	According to session plan

Table 1. WHO recommended storage temperatures and maximum storage periods of diphtheria containing vaccines in a country cold chain system (World Health Organization, 2011).

Since the diphtheria containing vaccines are sensitive to freezing, the vaccines should be protected from being exposed to freezing temperatures both during storage and transport. Use of frozen icepacks is the major source of freezing in transport. Although for years, organizations recommended conditioning of icepacks as the best practice to prevent freezing in cold boxes, serious compliance problems have been observed and reported in the field. In principle, if used with freeze-sensitive vaccines, icepacks should be fully conditioned before being placed in the cold box with the vaccines (World Health Organization, 2002a). In order to do so, the frozen 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 through listening to a slush noise. Conditioning requires both space and, more importantly, time, therefore patience. An area of approximately 1 m^2 is needed to condition 25 icepacks, a number usually required for loading one large cold box. This practice is generally found to be impractical and unrealistic because it requires more than one hour 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 (World Health Organization, 2004).

Although conditioning of frozen icepacks is said to be followed in the field, in a recent systematic review the occurrence of freezing temperatures during transport was found to be 16.7% in developed countries compared to 35.3% in developing countries. This difference is not statistically significant, potentially indicating that the current transport practice common to all countries – vaccines placed with frozen ice packs inside of insulated carriers – is placing vaccines at risk, regardless of the resource setting in which it is conducted (Matthias et al., 2007). In the six studies that analyzed the exposure of vaccine shipments to freezing temperatures as they travelled through both shipment and storage segments of the cold chain from either national or regional stores all the way to peripheral health centres, the findings were even more striking. In these studies, between 75% and 100% of the vaccine shipments were exposed to freezing temperatures at least once during the distribution process (Matthias et al., 2007). These comprehensive studies suggest that the risk of damaging freeze-sensitive vaccines is present in virtually every stage of the cold chain.

Between 2002 and 2004, WHO conducted a series of controlled laboratory studies and field tests (Nepal, Myanmar, Turkey and Zimbabwe) to assess the impact of using cool water packs (pre-cooled to a temperature between +2°C to +8°C) on the cold life of the vaccine transportation boxes and on the shelf life of the vaccines (Kartoğlu et al., 2009). Evaluations were conducted to verify the assumption that cool water packs can safely replace the use of icepacks for the transport of vaccines and, thus prevent the freezing of vaccines. Based on the recorded temperatures, the remaining shelf life of the vaccines were calculated through vaccine vial monitor (VVM) reactions using the Arrhenius equation[1]. Based on the results, investigators defined "cool life" (+2°C to +20°C) as a safety margin such that all vaccines except OPV can safely be transported with cool water packs even in hot climates and up to a repetition of four times (Kartoğlu et al., 2009). Fig 2 illustrates the impact of temperatures to vaccine shelf life calculated based on VVM reaction.

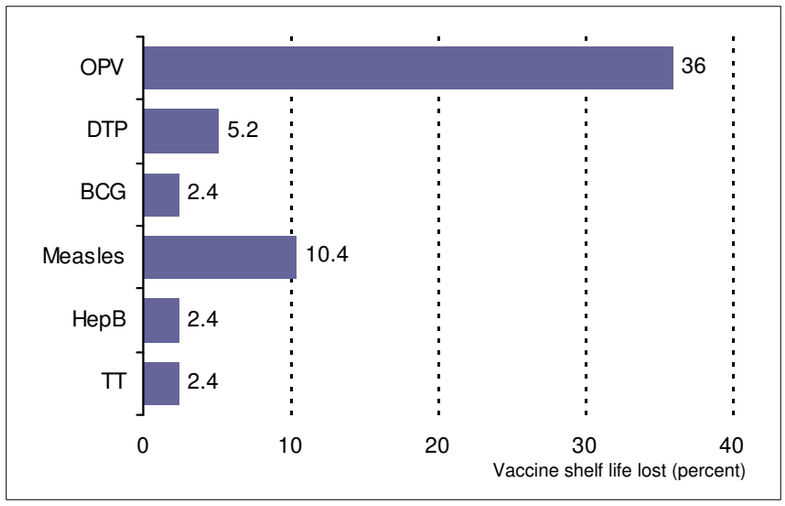


Fig. 2. Temperature impact on life loss of vaccines calculated on the basis of VVM reaction (Each transportation is assumed to be done at a continuous ambient temperature of +43°C for a period of 48 hours with a minimum temperature reading inside the vaccine transport box recorded as 11.5°C, a maximum of 25.3°C, and an average of 18.9°C throughout each journey. This scenario was repeated four times.)

Following this study, the Performance, Quality and Safety project at WHO has included the definition of "cool life" in passive cooling equipment performance specifications and now requires additional testing for cool life in prequalification of passive containers (World Health Organization, 2011a). Cool life (*with cool water-packs at +5°C)* 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 (World Health Organization, 2011a).

[1] The Arrhenius equation gives the quantitative basis of the relationship between the activation energy and the rate at which a reaction proceeds. Both VVM and vaccine degradation due to time and temperature exposure follow Arrhenius equation. For details on how a VVM works, please refer to section 3.2 Vaccine vial monitors and diphtheria containing vaccines.

The above results demonstrate that the use of cool water packs is a safe practice for vaccines, including diphtheria containing formulations. 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 vaccine transport system has been observed in Moldova during an assessment (Babalioğlu & Kartoğlu, 2004). 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. Special study protocols should be used for this particular purpose (World Health Organization, 2005).

3.2 Vaccine vial monitors (VVM) and diphtheria containing vaccines

A vaccine vial monitor (VVM) is a label containing a heat-sensitive material which is placed on a vaccine vial to register cumulative heat exposure over time (World Health Organization, 2002, 2011b, 2011c). The VVM, which was introduced in 1996 for Oral Polio Vaccine (OPV), became available for all other vaccines including diphtheria containing vaccines in 1999 (World Health Organization, 2005). Today, all diphtheria containing presentations come with VVM through the United Nations (UN) procurement agencies. VVM clearly indicates to health workers whether a vaccine can be used. VVM is designed to meet the vaccine's heat stability curve, allowing a margin of safety (World Health Organization, 2011b, 2011c). Correlation between the vaccine vial monitor and vaccine potency was tested with OPV and good correlation was found (World Health Organization, 1999b).

Photograph: PT Biofarma, Indonesia

Fig. 3. Vaccine vial monitor on Td vaccine (PT Biofarma, Indonesia).

The inner square of the VVM is made of heat sensitive material (monomer) that is light at the starting point and becomes darker with the combined effect of time and heat exposure. This change (polymerization) is cumulative and irreversible. Until the temperature and/or duration of heat reaches a level known to degrade the vaccine beyond acceptable limits, the inner square remains lighter than the outer circle. At the discard point, the inner square

reaches the same color as the outer circle. This reflects that the vial has been exposed to an unacceptable level of heat and the vaccine degraded beyond acceptable limits. The inner square will continue to darken with heat exposure until it is much darker than the outer circle. Whenever the inner square matches or is darker than the outer circle, the vial must be discarded.

The below Fig 4 explains the interpretation of VVM.

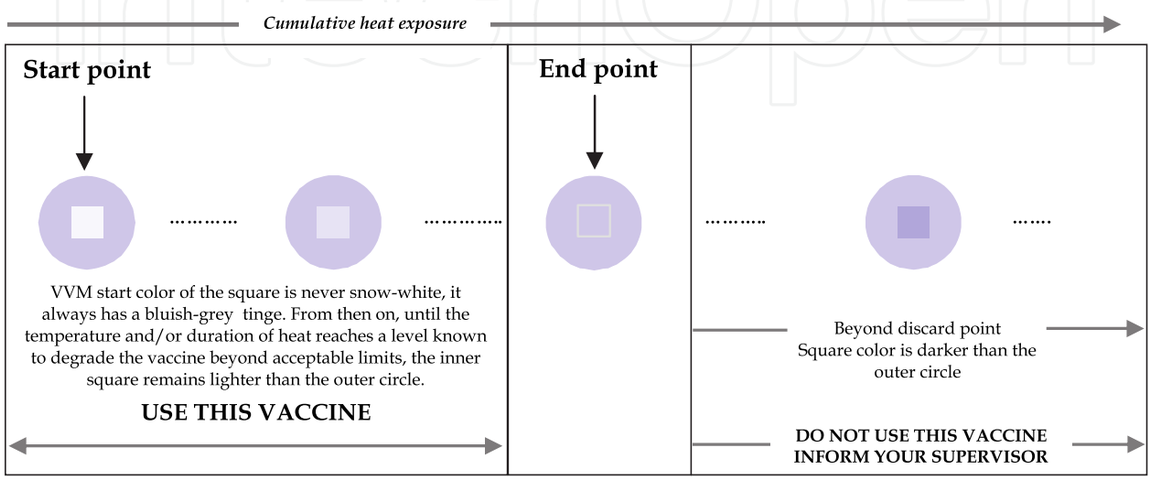


Fig. 4. VVM interpretation guidelines (Milstien et al., 2006).

A direct relationship exists between the rate of color change and temperature:

- The lower the temperature, the slower the color change.
- The higher the temperature, the faster the color change.

VVMs are located either on the label or on the top of the cap or on the neck of the ampoule depending on the following conditions. Diphtheria containing vaccines fall in the first category and VVMs in these vaccines are applied to their labels (World Health Organization, 2011b, 2011c):

- For multi-dose vials containing a vaccine that can be used in subsequent sessions: regardless of the vaccine presentation (liquid, freeze-dried or two vial combinations of liquid and freeze-dried), the VVM must be permanently attached to the label of the vaccine vial and must remain readily observable before, during, and after use, until the entire contents of the vial have been used.
- For vaccines that must be discarded at the end of the session or within 6 hours, whichever comes first: the VVM must be attached to the vaccine vial or ampoule and must remain readily observable until the vial or ampoule is opened, but not observable after opening. In order to achieve this requirement, the VVM must be located on the flip-off top of a vial or on the neck of an ampoule.

There are four different types of VVMs designed for different stability profiles (Table 2). Reaction rates are specific to four different models of VVM, relating to four groups of vaccines according to their heat stability at minimum two specific temperature points.

Category (Vaccines)	No. of days to end point at +37°C	No. of days to end point at +25°C	Time to end point at +5°C
VVM 30: High Stability	30	193	> 4 years
VVM 14: Medium Stability	14	90	> 3 years
VVM 7: Moderate Stability	7	45	> 2 years
VVM 2: Least Stable	2	N/A*	225 days

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

Table 2. VVM reaction rates by category of heat stability (World Health Organization, 2011b).

The above table does not give specific references to vaccine products, and only refer to the stability profile. Same type vaccines made by different manufacturers may have different heat stability characteristics and may therefore be assigned to different categories by WHO. In general, DT and Td combinations are either with VVM14 or VVM30 depending on their stability characteristics. DTP combination vaccines are usually with VVM14 mainly due to limiting component of pertussis.

Vaccines with VVMs including diphtheria containing ones can be taken out of the cold chain if health workers and others persons handling the vaccines have been trained to interpret VVM readings correctly and to discard any vial bearing a VVM that has reached its discard point. Although most of the out-of-cold chain studies are conducted with HepB vaccine and OPV, recent studies show that taking vaccines with VVMs out of the cold chain can successfully be implemented without compromising vaccine potency (Guthridge et al., 1996; Halm et al., 2010; Hipgrave et al., 2006; Huong et al., 2006; Lixia et al., 2007; Nelson et al., 2004; Otto et al., 2000; Zipursky et al., 2011). WHO recommends all Member States to consider adoption of policies permitting the use of vaccines beyond the cold chain where warranted for routine immunization activities or on a limited basis in certain areas or under special circumstances, such as (World Health Organization, 2007a):

- national immunization days;
- hard-to-reach geographical areas;
- immunizations provided at home - including hepatitis B vaccine birth dose;
- cool seasons;
- storage and transportation of freeze-sensitive vaccines (DTP, TT, DT, Td, hepatitis B and Hib vaccines) where the risk of freezing is greater than the risk of heat exposure.

In 2007, WHO has celebrated the 10 year anniversary of VVM introduction. Detailed information on the event as well as many other visuals and documents on VVM can be reached at http://www.who.int/immunization_standards/vaccine_quality/vvm_10years/en/index.html (World Health Organization, 2007b).

3.3 Shake test: detecting freeze-damage to diphtheria containing vaccines

Practices inadvertently exposing vaccines to sub-zero temperatures are widespread in both developed and developing countries and at all levels of health systems. (Bishai et al.,

1992; Burgess & McIntyre, 1999; Hanjeet et al., 1996; Lugosi & Battersby, 1990; Jeremijenko et al., 1996; Milhomme, 1993; Thakker & Woods, 1992; Wawryk et al., 1997; Wirkas et al., 2006). The most recent systematic literature review of vaccine freezing practices showed that inadvertent freezing occurs across all parts of the cold chain (Matthias et al., 2007). Between 14% and 35% of refrigerators or transport shipments were found to have exposed vaccines to freezing temperatures. In studies that all segments of the distribution chain were studied, between 75% and 100% of the vaccine shipments were exposed to sub-zero temperatures.

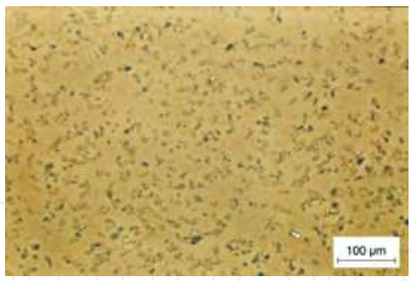
When a vaccine containing an antigen adsorbed to an aluminium adjuvant (e.g. hepatitis B, diphtheria toxoid, ..) is damaged by freezing, the loss of potency can never be restored, the damage is permanent (Dimayuga et al., 1995; World Health Organization, 1980).

Freezing affects the adsorbed vaccines by changing their physical form. Freezing does not affect non-potency parameters (such as acid content, pH; flocculating ability (Lf); ratio of free aluminium to aluminium phosphate; free formaldehyde; and thiomersal content). After freezing, the lattice (made up of bonds between the adsorbent and the antigen) in a vaccine is broken, whether monovalent or combined. Separated adsorbent tends to form larger, heavier granules that gradually settle at the bottom of the vial when this is shaken. It has been observed that ice crystals formed during freezing force aluminium particles to overcome repulsion, thereby producing strong inter-particle attraction resulting in aluminium particle coagulation/agglomeration. Thus the particles become bigger and heavier. As a simple physics rule, these heavy particles sediment faster than particles in never frozen vaccines. The size of the granules seems to increase on repeated freezing and thawing cycles.

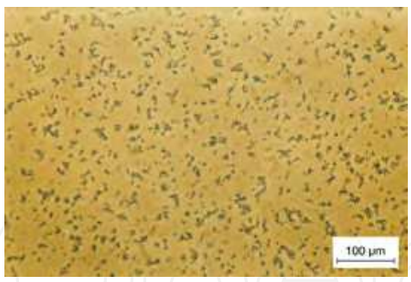
As shown in Fig 5, diphtheria containing vaccines kept at the optimal temperature (+2^{o}C to +8^{o}C) show a fine-grain structure under PCM. In contrast, large conglomerates of massed precipitates with a crystalline structure are observed in vaccines affected by freezing (Kartoğlu et al., 2010a). Vaccines that are exposed to subzero temperatures without freezing show identical physical characteristics to vaccines that are kept at optimum conditions. These vaccines were also found to be in full liquid state despite being exposed to -2^{o}C over a 24 hour period.

In this study, under PCM, particles in the non-frozen samples measured from 1 µm (DTP and DTP-HepB) to 20 µm (DT). By contrast, aggregates in the freeze-damaged samples measured up to 700 µm (DTP) and 350 µm on average (Kartoğlu et al., 2010a).

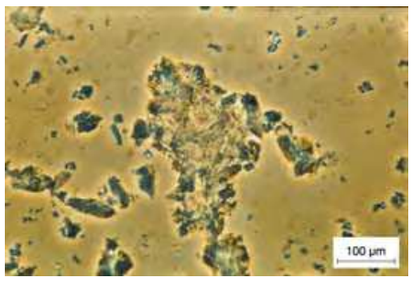
Scanning electron microscopy and X-ray analysis results in frozen and non-frozen diphtheria containing vaccines are illustrated in Fig 6, 7 and 8 (With permission from Kartoğlu, U., World Health Organization, Geneva, Switzerland and Kurzatkowski, W., Institute of Hygiene, Warsaw, Poland). Scanning electron microscopy of vaccines kept at +2°C to +8^{o}C showed uniform flocculent structure either dense or dispersed (Fig 6A). Scanning electron microscopy of vaccines damaged by freezing (exposed to -25 °C for 24 hours) exhibited conglomerates either with rough or smooth surfaces (Fig 7A and B). Phosphate content was found to be related with formation of the precipitates, lower values are mostly resulted in rough surfaces with sharp edges while higher phosphate content affected precipitates' surfaces to be more smooth. As shown in Fig 6B and 8B, X-ray analysis



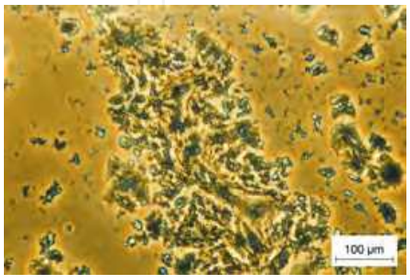
Fine-grain structure of dT vaccine kept between +2°C to +8°C temperature



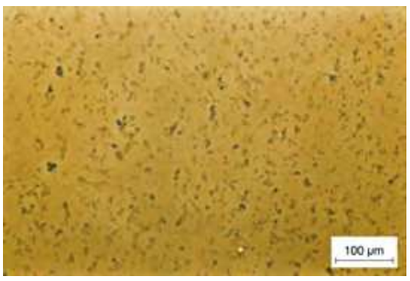
Fine-grain structure of DTP-HepB vaccine kept between +2°C to +8°C temperature



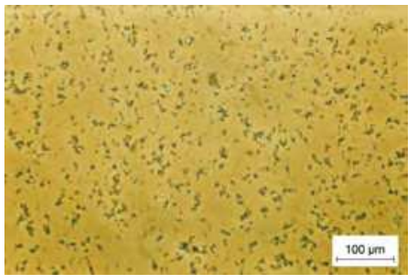
dT vaccine affected by freezing (-25°C) showing large conglomerates of large precipitates with crystalline structure



DTP-HepB vaccine affected by freezing (-25°C) showing large conglomerates of large precipitates with crystalline structure



Fine-grain structure in dT vaccine exposed to -2°C for 24 hrs

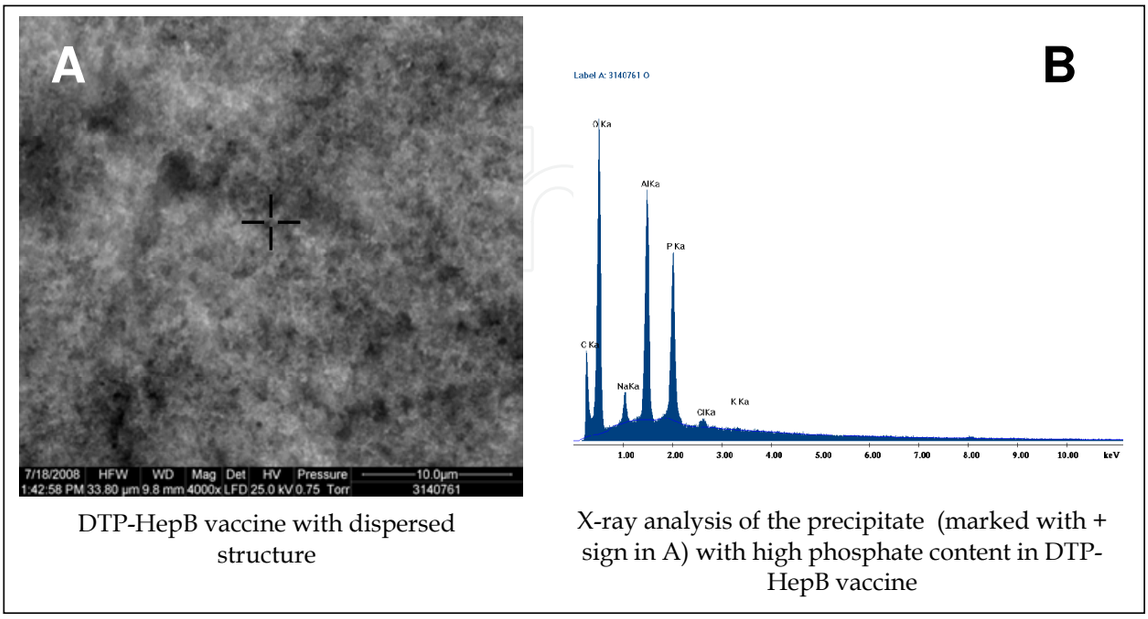


Fine-grain structure in DTP-HepB vaccine exposed to -2°C for 24 hrs

Fig. 5. Phase contrast microscopy of various vaccines kept at different temperatures (Kartoğlu et al., 2010a).

of precipitates in vaccines affected by freezing showed high aluminum content, indicating that the conglomerates are mainly aluminium clutters.

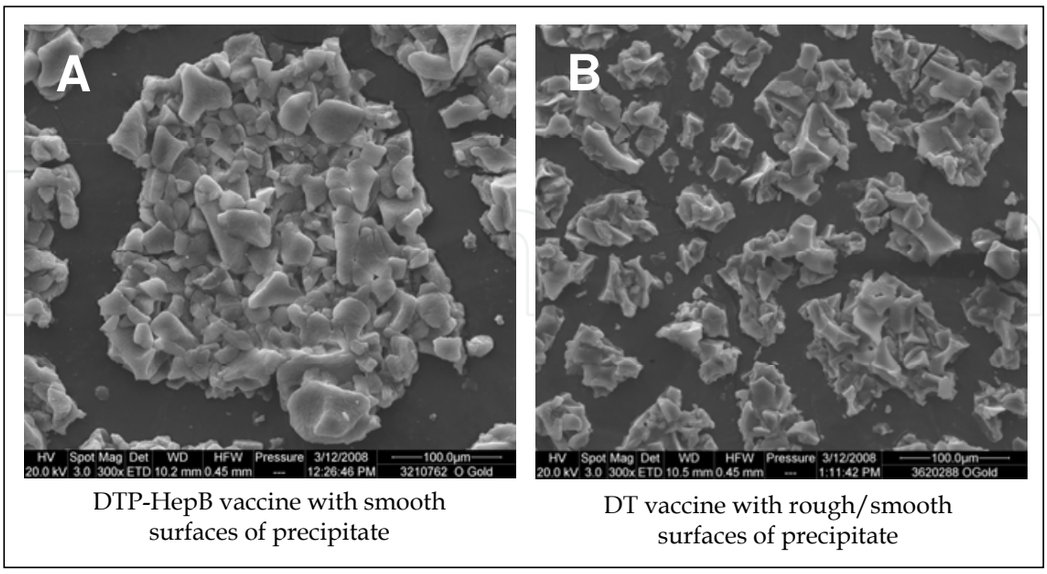
The physical changes initiated by freezing can be detected by the shake test simply by naked eyes. The shake test is designed to understand whether the vaccines are damaged by freezing based on the difference in sedimentation rates of freeze-sensitive vaccines in frozen and non-frozen vials (Fig 9). Shake test is validated by a WHO study against PCM with a 100% positive predictive value (Kartoğlu et al., 2010a, 2010b). In a typical demonstration of the shake test, two identical vials of a vaccine (i.e. from the same batch and the same manufacturer) that is suspected of having been exposed to freezing temperatures are selected; one of the two vials is purposely frozen and then thawed as the "negative control", while the second vial serves as the vial to be "tested" against this negative control. The two



DTP-HepB vaccine with dispersed structure

X-ray analysis of the precipitate (marked with + sign in A) with high phosphate content in DTP-HepB vaccine

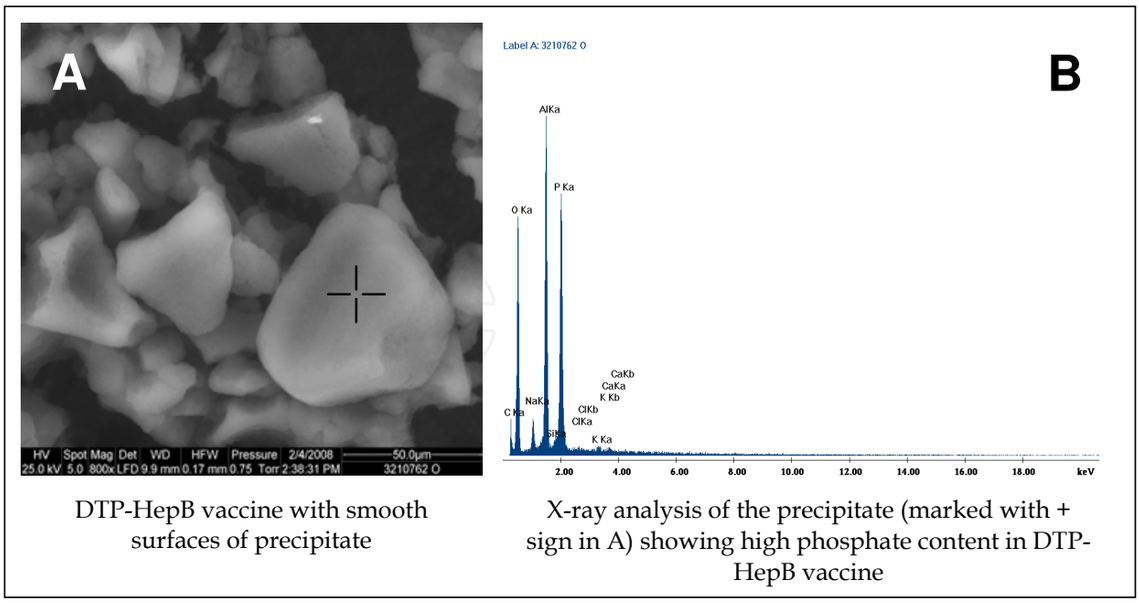
Fig. 6. Scanning electron micrograph (A) and X-ray analysis of the elements (B) of non-frozen DTP-HepB vaccine (kept at +2°C to +8°C at all times).



DTP-HepB vaccine with smooth surfaces of precipitate

DT vaccine with rough/smooth surfaces of precipitate

Fig. 7. Scanning electron micrographs of gold coated conglomerates of frozen DTP-HepB (A) and DT vaccines (B) exposed to -25°C for 24 hrs

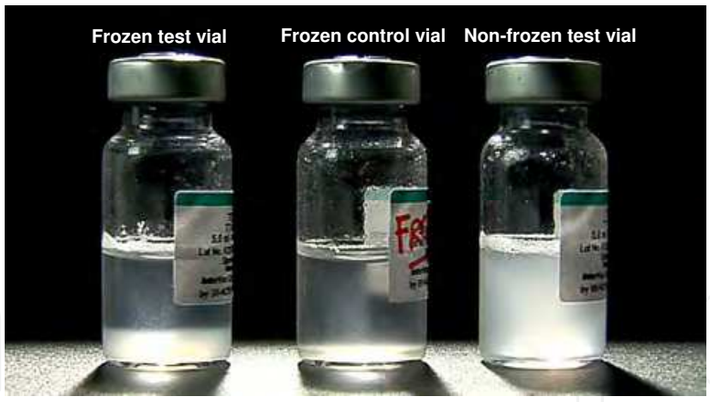


DTP-HepB vaccine with smooth surfaces of precipitate

X-ray analysis of the precipitate (marked with + sign in A) showing high phosphate content in DTP-HepB vaccine

Fig. 8. X-ray analysis of the elements of frozen DTP-HepB vaccine exposed to -25°C for 24 hrs.

vials are held together in one hand and shaken; they are then placed side by side on a flat surface. Provided the test vial has not been frozen, sedimentation is slower in the test vial than in the control vial that has been frozen and thawed. If the test vial has been frozen, the test and control vials will have similar sedimentation rates.



Photograph: Ü. Kwanç (WHO)

Fig. 9. Visual difference in sedimentation rates after shake test for detecting freeze damage to adsorbed DTP vaccine (Kartoğlu et al., 2010a).

The shake test correctly identifies if a vaccine has been affected by freezing 100% of the time (95% confidence interval, CI: 0.97–1.00) and it also correctly identifies if a vaccine has not been frozen 100% of the time (95% CI: 0.99–1.00). Sensitivity and specificity of the shake test for slushy vaccines were both calculated as 100% (sensitivity 95% CI: 0.86–1.00; specificity 95% CI: 0.93–1.00). In addition to the article (Kartoğlu et al., 2010a), WHO has produced a video article illustrating all steps of the validation study. This can be viewed at http://vimeo.com/8381355 (Kartoğlu et al., 2010b).

The shake test should not be conducted under the following circumstances and vials should be discarded immediately, without the need for any confirmatory shake test (Milstien et al., 2006):

- When a solid frozen vaccine vial(s) has been found
- With a vial for which a homogeneous solution CANNOT be obtained after vigorous shaking as seen in Fig 10. In such cases, the white lump/sediment cannot be separated from the walls of the glass vial. This happens only with DTP vials that are exposed to subzero temperatures without freezing (due to P component).

Photograph: Ü. Kartoğlu (WHO)

Fig. 10. Sub-zero temperature effect on DTP vaccine (after 10 minutes of vigorous shaking)

A learning guide to conduct the shake test is given in Box 1.

4. Summary

Diphtheria toxoids are some of the most stable vaccines in common use. They are stable at temperatures of 2 to 8°C for years, at room temperature for months, and at 37°C for weeks. At the temperature of 45°C the degradation of diphtheria toxoid is accelerated and its potency can decline during few weeks. At 53°C diphtheria toxoid lose its potency after few days, and at 60°C potency lost occurs within few hours. Freezing can reduce the potency of adsorbed diphtheria toxoid containing vaccines, however, it does not seem to affect the immunogenicity of unadsorbed products. The freezing point for adsorbed toxoids is between -5°C and -10°C. Adsorbed diphtheria toxoids containing vaccines should never be frozen.

As recommended by the WHO, all diphtheria toxoid containing vaccine products should be stored at +2°C to +8°C at all levels of any cold chain. Use of frozen icepacks at transport increases the risk of freezing the diphtheria containing vaccines. It has been observed and reported that the conditioning of icepacks for the purpose of preventing freezing during transport is not practiced in the field. Today WHO recommends to remove ice and introduce cool water packs (pre-cooled to a temperature between + 2°C to + 8°) for in-country transport of freeze-sensitive products including diphtheria containing vaccines.

Name of health staff: _____

Performance assessment scale:

1. Insufficient: Health staff performs the shake test incorrectly, or not in the right order or skips it altogether.

2. Competent: Health staff performs the shake test correctly and in the right order but either misses some points or needs to be reminded and encouraged by the study coordinator.

3. Proficient: Health staff performs the shake test correctly, in the right order, and without hesitating.

NOTES: □ **This protocol must not be altered**. There is only one correct way to conduct a Shake Test. □ The test procedure described below should be repeated with all suspect batches. In the case of international arrivals, the shake test should be conducted on a random sample of vaccine. However, if there is more than one lot in the shipment, the random sample must include a vial taken from each and every lot.		**Practice no.**		
		1	**2**	**3**
1. Take a vial of vaccine of the same type and batch number as the vaccine you want to test, and made by the same manufacturer.				
2. Clearly mark the vial as **"FROZEN"**.				
3. Freeze the vial in a freezer or the freezing compartment of a refrigerator until the contents are completely solid.				
4. Let it thaw. Do **NOT** heat it!				
5. Take your **"TEST"** vial from the batch that you suspect has been frozen.				
6. Hold the **"FROZEN"** vial and the **"TEST"** vial together in one hand.				
7. Shake both vials vigorously for 10-15 seconds.				
8. Place both vials on a flat surface side-by-side and start continuous observation of the vials until test is finished. *(NOTE: If the vials have large labels, which conceal the vial contents, turn both vials upside down and observe sedimentation in the neck of the vial.)*				
9. Use an adequate source of light to compare the sedimentation rates between vials. **IF,**				
10. The TEST vial sediments slower than the FROZEN vial, **THEN,**	10. Sedimentation is similar in both vials **OR** The TEST vial sediments faster than the FROZEN vial **THEN,**			
11. Use the vaccine batch.	11. Vaccine damaged: Notify your supervisor. Set aside all affected vaccine in a container marked "DAMAGED VACCINE FOR DISPOSAL– DO NOT USE"			
	12. Discard all affected vaccine once you have received permission to do so.			
	13. Fill in the Loss/Adjustment Form.			

Box 1. Shake test learning guide.

Heat impact on vaccines is cumulative. The VVM, which was introduced in 1996 for Oral Polio Vaccine (OPV), became available for all other vaccines in 1999. Today, all diphtheria containing products procured by the United Nations procurement agencies come with VVM. At any time in the process of distribution and at the time a vaccine is administered, the VVM indicates whether the vaccine has been exposed to a combination of excessive temperature over time and whether it is likely to have been damaged. It clearly indicates to health workers whether a vaccine can be used. With the help of VVM, vaccines can be taken beyond the cold chain under special circumstances defined by the WHO. These include national immunization days, hard-to-reach geographical areas; immunizations provided in the home - including hepatitis B vaccine birth dose; cool seasons; storage and transportation of freeze-sensitive vaccines (DTP, TT, DT, Td, hepatitis B and Hib vaccines) where the risk of freezing is greater than the risk of heat exposure.

Freezing of vaccines is a widespread problem across the world. When a vaccine containing an antigen adsorbed to an aluminium adjuvant is damaged, the loss of potency can never be restored. Freezing affects the physical form of the adsorbed vaccines through breaking the lattice structure that is made up of bonds between the adsorbent and the antigen. Separated aluminium adjuvant tends to form larger, heavier granules that gradually settle at the bottom of the vial when the latter is shaken. The shake test can demonstrate these facts and is the only test to determine whether freeze-sensitive adsorbed vaccines have been affected by freezing.

5. References

Aleksandrowicz, J.; Drozdz, M., Fieka, M. & Kurzatkowski, W. (1990). Evaluation of the physico-chemical state of aluminium hydroxide in biopreparations stored at various conditions. *Medycyna doswiadczalna i mikrobiologia,* Vol. 42, No.3-4, pp. 163-170, ISSN. 0025-8601

Babalioğlu, N. & Kartoğlu, U. (2004). *EVSM assessment: Chisinau primary vaccine store*, Republic of Moldova. 6-10 December 2004 (unpublished EVSM external assessment report)

Bishai, D.M.; Bhatt, S., Miller, L.T. & Hayden, G.F. (1992). Vaccine storage practices in pediatric offices. *Pediatrics* Vol. 89, No. 2, pp. 193–196, ISSN. 0031-4005

Burgess, M.A. & McIntyre, P.B. (1999). Vaccines and the cold chain: is it too hot... or too cold? *Med J Aust,* Vol. 171, No. 2, pp. 82, ISSN 0025-729X

Dimayuga, R.; Scheifele, D. & Bell, A. (1995). Effects of freezing on DTP and DTP-IPV vaccines, adsorbed. *Can Commun Dis Rep* Vol. 21, pp. 101–103, ISSN. 1188-4169

Guthridge, S.L. & Miller, N.C. (1996). Cold chain in a hot climate. *Australian and New Zealand Journal of Public Health.* Vol. 20, No. 6, pp. 657-660, ISSN. 1326-0200

Halm, A.; Yalcouyé, I., Kamissoko, M., Keïta, T., Modjirom, N., Zipursky, S., Kartoğlu, U. & Ronveaux, O. (2010). Using oral polio vaccine beyond the cold chain: a feasibility study conducted during the national immunization campaign in Mali. *Vaccine.* Vol. 28, No. 19, pp. 3467-3472.

Hanjeet, K.; Lye, M.S., Sinniah, M. & Schnur, A. (1996). Evaluation of cold chain monitoring in Kelantan, Malaysia. *Bull World Health Organ* Vol. 74, No. 4, pp. 391–397, ISSN. 0042-9686

Hipgrave, D.B.; Maynard, J.E. & Biggs, B.A. (2006). Improving birth dose coverage of hepatitis B vaccine. *Bulletin of the World Health Organization,* Vol. 84, No. 1, pp. 65–71, ISSN. 0042-9686

Huong, V.M.; Hipgrave, D., Hills, S., Nelson, C., Hien, D.S. & Cuong, N.V. (2006). *Out-of-Cold-Chain Delivery of the Hepatitis B Birth Dose in Four Districts of Vietnam*. PATH. October 2006 (unpublished study)

Jeremijenko, A.; Kelly, H., Sibthorpe, B. & Attewell, R. (1996). Improving vaccine storage in general practice refrigerators. *BMJ* Vol. 312, No.7047, pp. 1651–1652, ISSN. 0959-8138

Kartoğlu, U. (2007). Five senses, 14 September 2011, Available from: http://vimeo.com/8373523

Kartoğlu, U.; Ganivet, S., Guichard, S., Aiyer, V., Bollen, P., Maire, D. & Altay, B. (2009). Use of cool water packs to prevent freezing during vaccine transportation at the country level. *PDA Journal of Pharmaceutical Science and Technology.* Vol. 63, No. 1, pp. 11-26, ISSN. 1079-7440

Kartoğlu, U.; Ozguler, N.K., Wolfson, L.J. & Kurzątkowski, W. (2010). Validation of the shake test for detecting freeze damage to adsorbed vaccines. *Bull World Health Organ* Vol. 88, No. 8, pp. 624-631, ISSN. 0042-9686

Kartoğlu, U.; Ozguler, N.K. & Wolfson, L.J. (2010). Shake and tell Tell - video article. 14 September 2011, Available from: http://vimeo.com/8381355

Kartoğlu, U. (2010). Step-by-step how to conduct a shake test - educational video. 15 September 2011, Available from http://vimeo.com/8389435

Lixia, W.; Junhua, L., Haiping, C., Fangjun, L., Gregory, L.A., Carib, N., Wenyuan, Z. & Craig, N.S. (2007). Hepatitis B vaccination of newborn infants in rural China: evaluation of a village-based, out-of-cold-chain delivery strategy. *Bulletin of the World Health Organization,* Vol.85, No. 9, pp.688–694, ISSN. 0042-9686

Lugosi, L. & Battersby, A. (1990). Transport and storage of vaccines in Hungary: the first cold chain monitor study in Europe. *Bull World Health Organ* Vol. 68, No. 4, pp. 431–439, ISSN. 0042-9686

Matthias, D.M.; Robertson, J., Garrison, M.M., Newland, S. & Nelson, C. (2007). Freezing temperatures in the vaccine cold chain: a systematic literature review. *Vaccine* Vol. 25, No.20, pp. 3980–3986, ISSN. 0264-410X

Milhomme, P. (1993). Cold chain study: danger of freezing vaccines. *Can Commun Dis Rep* Vol. 19, No. 5, pp. 33–38, ISSN. 1188-4169

Milstien, J.; Kartoğlu, U. & Zaffran, M. (2006). *Temperature sensitivity of vaccines.* World Health Organization, WHO/IVB/06.10, Geneva, Switzerland

Nelson, C.M.; Wibisono, H., Purwanto, H., Manssur, I., Moniaga, V. & Widjaya, A. (2004). Hepatitis B vaccine freezing in the Indonesian cold chain: evidence and solutions. *Bulletin of the World Health Organization*, Vol. 82, No. 2, pp. 99-105, ISSN. 0042-9686

Otto, B.F.; Suarnawa, I.M., Steward, T., Nelson, C., Ruff, T.A., Widjaya, A. & Maynard, J.E. (2000). At-birth immunisation against hepatitis B using a novel pre-filled immunisation device stored outside the cold chain. *Vaccine.* Vol. 18, No. 5-6, pp. 498-502, ISSN. 0264-410X

Serum Institute of India. (2005). Personal communication.

Shmelyova, E.I. (1976). Study of stability of physical properties and biological activity of liquid and freeze dried adsorbed pertussis-diphtheria-tetanus vaccines. *Proceedings of the symposium on stability and effectiveness of measles, poliomyelitis and pertussis vaccines.* Yugoslav Academy of Sciences and Arts, pp. 159-179, Zagreb, Yugoslavia

Sporzynska, Z. (1965). Studies on the stability of toxoids. I. The effect of temperature on the immunogenic properties of diphtheria toxoid. *Experimental medicine and microbiology*, Vol. 17, pp. 130-139.

Thakker, Y. & Woods, S. (1992). Storage of vaccines in the community: weak link in the cold chain? *BMJ* Vol. 304, No. 6829, pp. 756–758, ISSN. 0959-8138

Wawryk, A.; Mavromatis, C. & Gold, M. (1997). Electronic monitoring of vaccine cold chain in a metropolitan area. *BMJ* Vol. 315, No. 7107, pp. 518, ISSN. 0959-8138

World Health Organization. (1980). The effects of freezing on the appearance, potency, and toxicity of adsorbed and unadsorbed DTP vaccines. *Wkly Epidemiol Rec* Vol. 55, No. 50, pp. 385–392, ISSN. 0049-8414

World Health Organization. (1990). Tests of the freezing point of vaccines. *Cold chain newsletter*, 90.3, pp.5

World Health Organization. (1999). *Quality of the cold chain: WHO-UNICEF policy statement on the use of vaccine vial monitors in immunization services.* WHO/V&B/99.18, World Health Organization, Geneva, Switzerland

World Health Organization. (1999). *Testing the correlation between vaccine vial monitors and vaccine potency.* World Health Organization, WHO/V&B/99.11, Geneva, Switzerland.

World Health Organization. (2002). *Guideline for establishing or improving primary and intermediate vaccine stores.* WHO/V&B/02.34, World Health Organization, Geneva, Switzerland

World Health Organization. (2022). *Getting started with Vaccine Vial Monitors.* WHO/V&B/02.35, World Health Organization, Geneva, Switzerland

World Health Organization. (2004). *Immunization in Practice.* WHO/IVB/04.06, World Health Organization, Geneva, Switzerland

World Health Organization. (2005). *Study protocol for temperature monitoring in the vaccine cold chain*. WHO/IVB/05.01, World Health Organization, Geneva, Switzerland

World Health Organization. (2007). *WHO-UNICEF policy statement on the implementation of vaccine vial monitors: The role of vaccine vial monitors in improving access to immunization.* WHO/IVB/07.04, World Health Organization, Geneva, Switzerland

World Health Organization. (2007). WHO celebrates 10 years of VVM implementation, In: Immunization standards, 14 September 2011, Available from: http://www.who.int /immunization_standards/vaccine_quality/vvm_10years_index/en/index.html

World Health Organization. (2010). Effective vaccine management (EVM) initiative, In: *EVM - setting standards for the Immunization Supply Chain*, 14 September 2011, Available from: http:// www.who.int/immunization_delivery/systems_policy/logistics/ en/index6.html

World Health Organization. (2011). *Performance Quality and Safety Devices Catalogue*, (version 2 August 2011), pp. 1-234, Geneva, Switzerland, Retrieved from

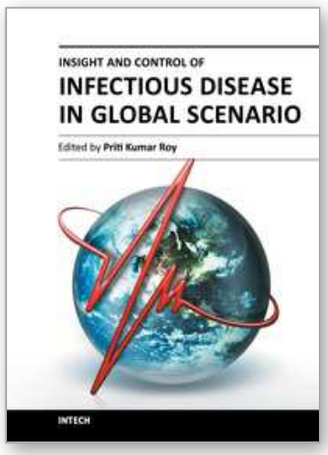
http://www.who.int/entity/immunization_standards/vaccine_quality/pqs_devices_catalogue_02aug2011.pdf

World Health Organization. (2011). *PQS performance specification for vaccine vial monitor.* Retrieved from WHO/PQS/E06/IN05.2, http://www.who.int/entity/immunization_standards/vaccine_quality/who_pqs_e06_in05_rev_july2011.pdf

World Health Organization. (2011). *PQS independent type-testing protocol for vaccine vial monitors.* Retrieved from WHO/PQS/E06/IN05.VP2, http://www.who.int/ entity/ immunization_standards/vaccine_quality/who_pqs_e06_in05_vp_rev_may2011.pdf

Wirkas, T.; Toikilik, S., Miller, N., Morgan, C. & Clements, C.J. (2006). A vaccine cold chain freezing study in PNG highlights technology needs for hot climate countries. *Vaccine* Vol. 25, No. 4, pp. 691–697, ISSN. 0264-410X

Zipursky, S.; Boualam, L., Cheikh, D.O., Fournier-Caruana, J., Hamid, D., Jannsen, M., Kartoğlu, U., Waeterlos, G. & Ronveaux, O. (2011). Assessing the potency of oral polio vaccine kept outside of the cold chain during national immunization campaign in Chad. *Vaccine.* Vol. 29, No.34 , pp. 5662-5656, ISSN. 0264-410X



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