WHO/GPV/98.07
ORIGINAL: ENGLISH
DISTR.: GENERAL

Thermostability of vaccines

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GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION

World Health Organization
Geneva
1998

The Global Programme for Vaccines and Immunization thanks the donors whose unspecified financial support has made the production of this document possible.

This document revises and replaces the 1989 document *Stability of vaccines*, WHO/EPI/GEN/89.08

Ordering code: WHO/GPV/98.07
Printed : December 1998

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List of abbreviations

ADT	accelerated degradation test
BC	benzethonium chloride
BCG	bacille Calmette-Guérin (vaccine)
CO_2	carbon dioxide
CP	culturable particle
D_2O	heavy water
DEV	duck embryo vaccine
DNA	deoxyribonucleic acid
DT	diptheria-tetanus vaccine
DTP	diphtheria-tetanus-pertussis vaccine
EPI	Expanded Programme on Immunization (WHO)
HB	hepatitis B
HbsAg	hepatitis B surface antigen
HDCV	human diploid cell vaccine
IPV	inactivated polio vaccine
IU/ml	international units per millilitre
JE	Japanese encephalitis
$MgCL_2$	magnesium chloride
MMR	measles, mumps, rubella (vaccine)
OPV	oral polio vaccine
PDEV	purified duck embryo vaccine
PFU	plaque-forming unit
PHK	primary Syrian hamster kidney cell culture
PVRV	vero cell line vaccine
TT	tetanus toxoid
VVM	vaccine vial monitor

Introduction

Every year the immunization services in developing countries prevent about 490 000 children from becoming paralysed by poliomyelitis. Over three million deaths are similarly prevented from measles, neonatal tetanus and pertussis (*51*). These achievements are partly attributable to the training of staff in the proper storage and transport of vaccines and partly to improvements in the cold chain.

However, vaccines are still not being stored and transported properly in many areas. Questions are often raised as to what should be done with stocks of vaccines that have been exposed for varying periods to elevated temperatures. There is no simple and cheap method that can be used in the field to assess whether a vaccine exposed to ambient temperature has retained at least the minimum required potency, although the vaccine vial monitors (VVMs) now provided with oral poliomyelitis vaccine (OPV) can indicate the level of heat exposure of individual vials. Vaccine potency can be determined only by costly laboratory assays, the results of which are often delayed for several months. Only a large number of doses can justify sending a vaccine for retesting (from 2000 doses for poliomyelitis and measles vaccines, to 200 000 doses for diphtheria-tetanus-pertussis (DTP) vaccine) (*45*).

A knowledge of a vaccine's stability, especially of the rate of decline in potency at a given temperature, can be helpful in determining storage requirements. The present document updates previously reported information on this subject (*54*), with particular regard to the stability of vaccines stored and transported at ambient temperatures or exposed to freezing temperatures.

In part two of the document, the stability of individual vaccines is analysed, dealing in the first section with the vaccines most commonly used in national immunization programmes, ranging from those that are highly stable, such as the toxoids, to the least stable, such as oral poliomyelitis vaccine. The following sections look at other viral and bacterial vaccines which are not yet broadly used, or which address diseases which are of regional rather than global public health importance.

Part I: Issues relating to vaccine preservation

1. Importance of the cold chain

To ensure the optimal potency of vaccines, storage and handling need careful attention. Adequate electrical power and refrigeration are often lacking in developing countries, where storage, handling and the heat stability of vaccines are consequently matters of great concern (*28*). New products have been developed for safe transport and storage, while the reliability of vaccine supply has been increased by the introduction of improved management techniques. Extensive training ensures that everyone involved in the cold chain is familiar with all its facets. However, evaluations in India (*47, 139*), Malaysia (*62*), Nepal (*46*), the United Republic of Tanzania (*131*), and Tunisia (*43*) showed that there were still weak points in cold chain performance and that more attention should be paid to it, especially in peripheral facilities.

The importance of maintaining the cold chain has been given little consideration in temperate countries. Although adequate refrigeration is often taken for granted, errors in vaccine handling may occur more commonly than is generally assumed (*21*). Substantial drops in vaccine potency caused by unsatisfactory conditions of delivery and storage have been reported (*84, 89*).

The most common deficiencies in cold chain performance reported from developed countries are: high temperatures during storage or transport (*21, 27, 90, 107, 138*); exposure of adsorbed vaccine to freezing temperatures (*64, 76, 107*); refrigerators without thermometers; failure to take and record temperature readings regularly (*17, 21, 39, 63, 68, 90, 138, 142*); storage of drugs, drinks, food and pathology specimens with vaccines (*21, 90*); and failure to discard unused vaccine after sessions at ambient temperature (*39*).

A high prevalence of avoidable errors was found in the metropolitan area of Los Angeles in the United States of America, where little attention was given to self-monitoring of vaccine storage practices (*21*). Studies in Hungary (*93*), Poland (*94*), and the United Kingdom of Great Britain and Northern Ireland (*23*) showed considerable weakness in the cold chain during vaccine transportation from manufacturers or from central distribution points to health clinics. In Hungary, cold chain monitors showed that at least 6% of DTP and 30% of BCG vaccines were exposed to excessive heat when carried by the postal service during the summer; in the winter, 38% of DTP vaccine consignments were exposed to freezing temperatures (*93*). In Poland, vaccine storage was generally satisfactory in central storehouses

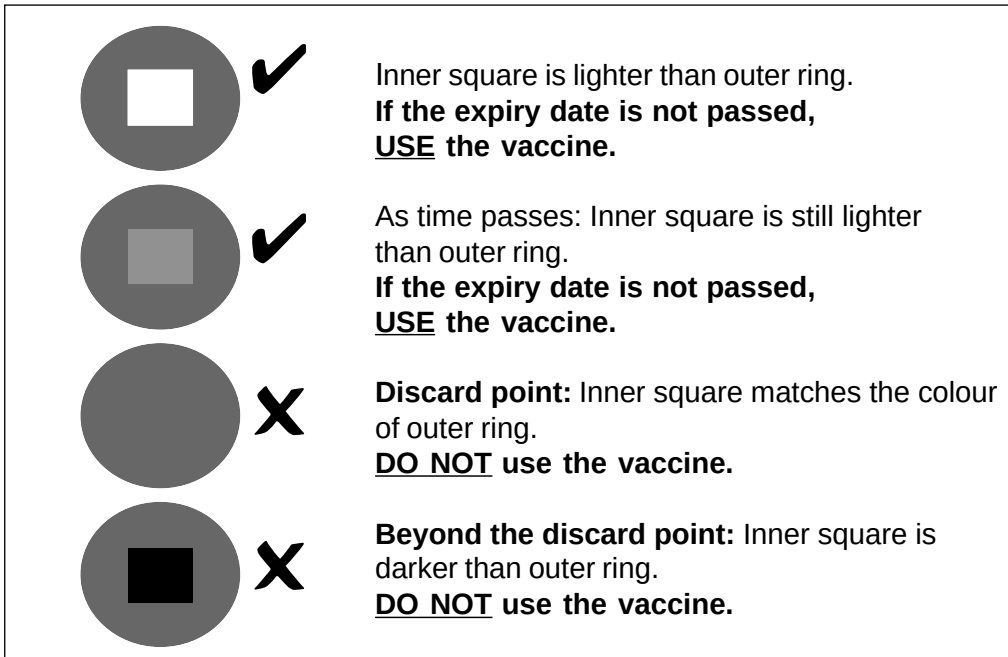
and sanitary epidemiological stations but was less so in outpatient clinics and health centres. Conditions for the transportation of vaccines were completely unsatisfactory at all levels (*94*).

2. Vaccine vial monitors and the future of the cold chain

Vaccine vial monitors, which measure exposure to heat, are time- and temperature-sensitive labels attached to vials of vaccine at the time of manufacture. Through a gradual colour change they warn health workers and storekeepers when a vaccine has been excessively exposed to heat and should not be used. They are designed to meet the vaccine's heat stability curve, allowing a margin of safety (*160*). They comply either with WHO requirements for heat stability or with heat stability data provided by the vaccine manufacturer if these are more stringent.

The information delivered by a VVM is simple. If the inner square is a lighter colour than the outer reference ring then the vaccine can be used. If the inner square is the same colour as the outer ring or darker than it, then the vaccine should not be used (Figure 1).

Figure 1: Vaccine vial monitor showing four stages of exposure



VVMs were first introduced on OPV vials supplied to UNICEF and WHO during the first quarter of 1996. They are part of the UNICEF and WHO tender specifications. Countries procuring vaccines directly are also beginning to request VVMs on OPV.

When the management and infrastructure of the Expanded Programme on Immunization (EPI) were being established it was impossible to check whether vaccines retained adequate potency during distribution. Consequently, for the past 20 years, vaccine cold chain systems have been built and maintained on the basis of a single set of rules governing vaccine-handling worldwide, without specific consideration of local environments and types of vaccine. The approach had the merit of simplicity, making the cold chain easy to understand, implement and manage, and presented an uncontroversial concrete objective to be achieved.

However, this approach has led to the gradual emergence of a dogmatic view of the cold chain, preventing health workers from taking full advantage of the actual heat stabilities of different vaccines.

Immunization programmes have now evolved and diversified: operational strategy reaches out to areas that are difficult to access, large target populations are covered in special campaigns, and a major effort is made to cover every unprotected child. Vaccines have become more stable and there is a clear prospect of increased or even complete heat stability. In these circumstances the dogmatic approach to the cold chain causes resources to be wasted and places unnecessary restrictions on field operations.

The VVM can be seen as a catalyst for much-needed changes in strategies of vaccine distribution via the cold chain. It should eventually allow immunization programmes to exploit the stability of each vaccine to the greatest possible extent, minimize distribution costs, and increase flexibility in the handling of vaccines in the field, thus helping to make operations more effective.

3. Methods for determining impact of heat or freezing on vaccine potency

Some authors have sought to determine the validity period of a vaccine by estimating loss of potency during long periods of storage at different temperatures. The accelerated degradation test (ADT) is more convenient. In this test samples are subjected to a range of elevated temperatures at which significant and readily detectable degradation is induced in a relatively short time. The rate at which it occurs is measured and extrapolation is made to the lower temperatures at which vaccines are stored, in accordance with the Arrhenius equation (*144*). The precision with which the ADT predicts degradation rates differs considerably, depending on the range of temperatures used, the number of samples tested and the design of the test. The use of ADT results may be further complicated by the different methods and techniques used for estimating the potency of vaccines.

The determination of virus titres of live attenuated vaccines against poliomyelitis, measles or rubella is a simple procedure. In contrast, the biological assays of bacterial vaccines and toxoids are difficult tests requiring large numbers of animals. Potency is expressed in arbitrarily established units or in effective doses providing 50% protection. The results of these tests are often subject to wide biological variation and it is difficult to obtain precise data on vaccine deterioration unless it has been substantial (*120*).

Vaccines and toxoids are made up of proteins, nucleic acids, lipids and carbohydrates, which undergo changes on exposure to heat. The degradation rate of a vaccine is determined by the storage temperature: the higher the temperature, the more rapid and extensive is the degradation. There are considerable differences between degradation rates. However, the degradation rate (b) is not the only factor determining the residual potency (Y_t) of a vaccine: the time (T) for which a vaccine is stored at a given temperature and the initial potency of the vaccine (Y_o) also have an influence.

The relationship between the three factors is expressed as follows:

$$Y_t = Y_o - bT$$

The usefulness of this formula is limited because many of those involved in immunization programmes may not know the initial potency of a vaccine. However, knowledge of the degradation rate characteristics for various temperatures and of the time of exposure of a suspect vaccine to a given temperature may help a health worker to decide what to do with it.

This document reviews current information on the stability of vaccines so that they may be used to their fullest potential.

Part II: Analysis of vaccine stability – vaccines commonly used in immunization programmes

4. Diphtheria and tetanus toxoids

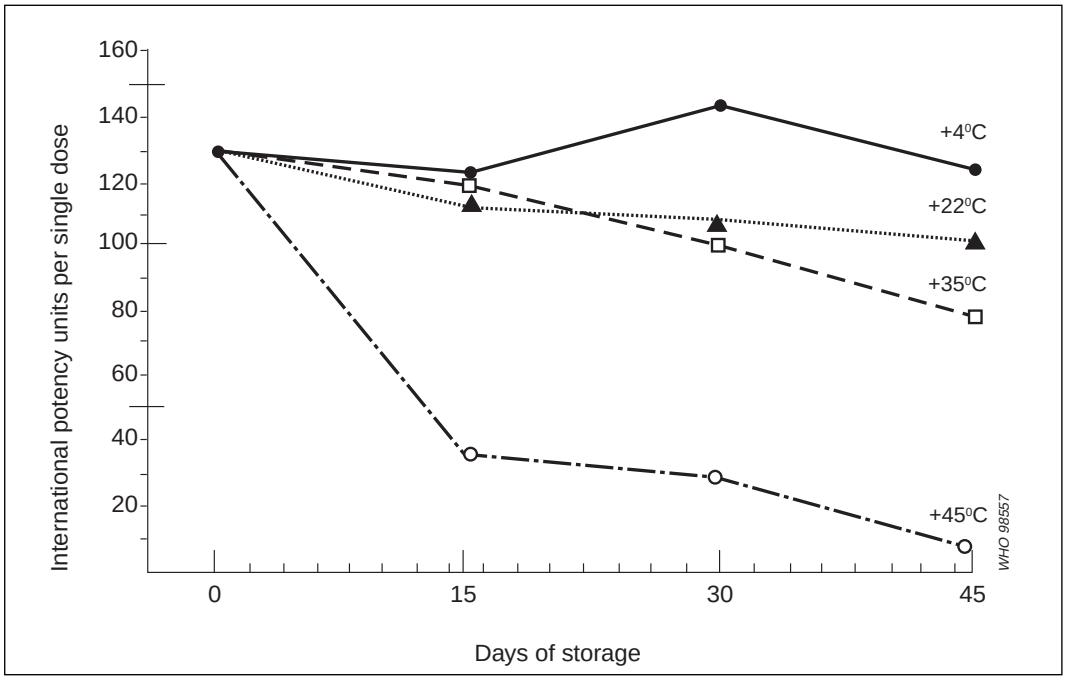
4.1 Exposure to high temperatures

Adsorbed diphtheria and tetanus toxoids in monovalent form or as components of combined vaccines are the most stable of the vaccines commonly used in national immunization programmes. Being proteins, they are generally subject to the rules governing temperature stability in this class of substance. They are stable at elevated temperatures, even for long periods of storage, but may change their appearance and lose potency when frozen. This is not because of the characteristics of the toxoids themselves but because an aluminum-based adjuvant with a particular gel structure is destroyed by freezing.

The potency of tetanus components of adsorbed DTP or DTP-poliomyelitis vaccines does not show significant change at temperatures in the range 4°C to 8°C for three to seven years (*80, 83, 130, 146*). The shelf-life, at the temperatures usually recommended by manufacturers (2°C to 8°C), depends on the nature of the vaccine: the validity period is usually longer for monovalent toxoids or combined diphtheria-tetanus vaccine (usually three years) than for DTP or DTP-polio vaccines (18 to 24 months). In combined vaccines the limiting factors are the pertussis or inactivated poliomyelitis components.

The toxoid components of DTP or DTP-polio vaccines show an insignificant decrease in potency when stored for 1.5 years at 18°C (*130*), for 6 to 12 months at 24°C (*136*), and for 2 to 6 months at 37°C (*83, 136, 146*). Other studies, however, have shown higher heat lability. Storage at 37°C for up to 22 weeks caused a 50% reduction in the potency of the diphtheria and tetanus components of DTP-polio vaccines of one producer (*146*). In some other DTP vaccines the tetanus toxoid component showed even more accentuated deterioration when stored for 45 days at 22°C and 35°C; the daily losses were about 0.5% and 1% respectively (Figure 2) (*85*).

Figure 2: Potency of tetanus component of DTP vaccine stored for 45 days at various temperatures



Source: Kumar V et al. (85)

The diphtheria and tetanus toxoid components of some DTP vaccines can withstand exposure to temperatures above 37°C lasting several weeks. No significant loss of potency was observed in either toxoid component when DTP vaccine from one producer was stored at 45°C for two to four weeks. However, storage at that temperature for eight weeks produced a loss in potency of about 40% (*83*). In some DTP vaccines the deterioration process was more rapid: at 45°C the loss in potency of the tetanus component was 5% per day in the first two weeks of storage and 1% per day during the next month (*85*).

At temperatures above 45°C the degradation of toxoid potency is accelerated, and Arrhenius behaviour no longer occurs as the proteins are denatured. After exposures to 53°C lasting four and eight days respectively, monovalent adsorbed tetanus toxoid subjected to the ADT lost 17% and 47% of its initial potency (Table 1) (*30*). Tetanus and diphtheria toxoids exposed to 60°C are destroyed in three to five hours (*30, 135*).

Table 1: Potency of adsorbed tetanus toxoid after various periods of storage at different temperatures

Temperature (°C)	Time of exposure (hours)	Remaining potency (%)
53	96	83
	192	53
55	32	97
	72	52
	144	44
	288	35
65	3	20

*Source: Cohen H, van Ramshorst JD, Tasman A (*30*).*

Adsorbed diphtheria and tetanus toxoids tested in Poland (*2*) showed a 50% decline in potency (half life) after 4 to 8 days at 53–55°C, after 80 to 90 days at 45°C, after 10 to 13 months at 35–37°C, and after 5 to 7 years at 20–25°C. The authors concluded that toxoids which were unintentionally left at room temperature up to two weeks can safely be used in humans without control potency testing.

4.2 Exposure to freezing temperatures

Freezing can reduce the potency of tetanus toxoid to an extent that evidently varies slightly with 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 between -5°C and -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 (*42*). This difference is undoubtedly due to the 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.

Frozen monovalent tetanus toxoid, especially that frozen four times, stimulated a lower mean response and a lower proportion of high titres than the unfrozen product in young military recruits, although the significance of the differences was unclear. All persons immunized with frozen toxoids, however, acquired protective levels of tetanus antitoxin. Freezing did not seem to affect the immunogenicity of unadsorbed toxoid (which remained less immunogenic than the adsorbed product) (Table 2) (*106*).

Table 2: Immune response of military recruits immunized with frozen and unfrozen adsorbed tetanus toxoid

Toxoid	Treatment	10 days after first dose		10 days after second dose		10 days after third dose	
		% >0.01 IU/ml	Mean in IU/ml	% >0.01 IU/ml	Mean in IU/ml	% >0.01 IU/ml	Mean in IU/ml
Adsorbed on $AlPO_4$	Unfrozen	50	0.07	89	4.0	90	13.5
	Frozen 1x	47	0.07	84	3.0	73	9.7
	Frozen 4x	46	0.05	77	2.4	69	9.2
Non-Adsorbed	Unfrozen	50	0.04	27	0.6	21	3.2
	Frozen 1x	50	0.05	36	0.7	34	3.3
	Frozen 4x	54	0.06	30	0.7	21	3.2

*Source: Menon PS et al. (*106*)*

4.3 Physical changes in aluminum adjuvant at high and low temperatures

The observations discussed above refer to the potency of toxoids as determined in animal tests. However, long exposure to high temperature may result in some changes in the physical characteristics of the aluminum compound which are not revealed by animal potency tests. The aluminum hydroxide adjuvant showed evidence of 'ageing', in the form of morphological and structural changes, when stored as a single compound or as the adjuvant of diphtheria, DT and DTP vaccines (*3, 163, 164*). A continuous decline in its ability to adsorb Congo red dye was observed during storage at temperatures of 4 to 10°C for 5.5 years. Electron microscope and roentgenographic studies showed that morphological and structural changes progressed more rapidly at 10°C than at 4°C (*163, 164*).

The freezing point for adsorbed DTP vaccines is between -5°C and -10°C (*42*). The time required to freeze DTP, DT or tetanus toxoid (TT) vaccines depends on the number of doses in the vial (the greater the volume, the longer the time) and on the temperature: 110 to 130 minutes at -10°C, 25 to 45 minutes at -20°C, and 9 to 11 minutes at -70°C. Because of supercooling, the temperature in DTP, DT or TT 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 (*50, 103*).

Adsorbed vaccines, whether monovalent or combined, alter their physical appearance after freezing has induced changes in the structure and morphology of the adsorbent. Changes in pH and storage at higher temperatures have no influence on the structure of aluminum gel, but freezing causes extensive morphological changes that are visible under the electron microscope (*3*). The development of agglomerates, floccules or other granular matter produces an increase in sedimentation rates (*3, 6, 42, 106, 130*), and the granules do not form a uniform suspension even on vigorous shaking. The size of the granules seems to increase on repeated freezing and thawing (*106*).

The physical changes induced by freezing provide the basis for the shake test, which can be useful in detecting previous freezing in adsorbed vaccines (*42*). This test is easy to perform: the vaccine container is vigorously shaken, the contents are examined for physical changes, and the extent of sedimentation is checked after 30 minutes. The presence of granular forms or floccules when shaking is effected, or the formation of a sediment at the bottom of the container within 30 minutes, with clear liquid above, suggest that the vaccine has been frozen. However, performing the test needs some experience. Furthermore, not all vaccines show physical changes after freezing.

If it is suspected that adsorbed DTP, DT, TT or hepatitis B vaccines have been frozen they should be examined for physical changes. Where these are found the vaccines should be discarded. The amount of antigen in a non-homogeneous vaccine can vary greatly, and the administration of such a vaccine may be associated with a reduced immune response or an increased incidence of local reactions.

4.4 Summary

Diphtheria and tetanus 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 toxoids is accelerated and their potency can decline during a few weeks. At 53°C toxoids lose potency after few days, and at 60°C they lose potency after just a few hours. Freezing can reduce the potency of adsorbed toxoids, 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 toxoids should never be frozen.

5. Hepatitis B vaccine

5.1 Exposure to low and high temperatures

Hepatitis B (HB) vaccine is a liquid suspension consisting of purified hepatitis B surface antigen (HBsAg) adsorbed on aluminium salt. A plasma-derived vaccine and a DNA recombinant vaccine are on the market. As with other vaccines adsorbed on aluminium salts, freezing of HB vaccine may cause significant reductions in potency. The risk of lost potency due to freezing may increase at the end of the cold chain when vaccine is transported in cold boxes and may come into close contact with cold packs. HB vaccine should be protected from being frozen; vaccine thought to have been frozen should not be used. The freezing point of HB vaccine is about -0.5°C.

At temperatures of 2 to 8°C, HB vaccine appears to be stable for many years. The upper limits of storage life have not been defined.

A yeast-derived recombinant DNA HB vaccine (Engerix-B) is apparently stable at elevated temperatures. The manufacturer considers it to be so for 30 days at 20–25°C, for one week at 37°C, and for three days at 45°C, the corresponding half-lives being calculated as nine months, 31 days and 13 days.

There were no differences in immune responses between healthy persons immunized with a recombinant vaccine heated to 37°C for one week and similar persons given a control vaccine stored at 4°C; the antibody distribution and geometric mean antibody titres were similar in the two groups. The total incidence, severity and types of symptoms were similar in persons immunized with the two vaccines, and no severe reactions were reported (*78*).

In another study, recombinant vaccine was studied in healthy volunteers. Using vaccine stored at 4°C for purposes of comparison it was found that heating vaccine for one week at 45°C or for one month at 37°C did not alter reactogenicity or the ability of the vaccine to elicit antibody titres considered protective (*145*).

Five manufacturers provided data on heat stability in their HB vaccines when they were stored at different temperatures. The vaccines tested represented routine production lots: four of plasma-derived products and one of recombinant vaccine (Table 3).

The results show that HB vaccines stored at 2°C to 8°C are quite stable for up to four years. The data revealed considerable differences between vaccines stored at elevated temperatures. At room temperatures of 20°C to 26°C, vaccines from three manufacturers were stable for at least one year, while the vaccine from another lost 50% of its initial potency in four months. At temperatures of 36°C to 40°C the vaccine from the fifth manufacturer had a lower level of heat stability than the others. The physical appearance, pH, content of merthiolate, aluminium and protein, sterility, and abnormal toxicity were unchanged in vaccines from one of the manufacturers during storage for 24 months at temperatures ranging from 2 °C to 36°C.

Table 3: Stability of five hepatitis B vaccines as indicated by immunogenicity tests on animals

Vaccine tested	Parameters used in testing vaccines	Storage temperature (°C)			
		2 - 8	20 - 26	36 - 40	45
A (plasma)	Longest storage without significant loss of potency	24 months	12 months	3 months	-
C (plasma)	Remaining potency (%) after specified time	100% after 24	70% after 24	70% after 24	-
D (plasma)	Longest storage with relative potency upper confidence interval >1	44 months	-	-	-
E (recombinant)	Longest storage with relative potency upper confidence interval >1	53 months	12 months	7 months	1 month
	Half life*	-	9 months	1 month	3 days
F (plasma)	Half life	>3 years	4 months	7 days	-

* Half life: time at which 50% loss of original potency occurs

Source: Manufacturers' data.

5.2 HB vaccine used outside the cold chain

A study was conducted in China to assess whether HB vaccine stored outside the cold chain for up to three months could remain effective and still be delivered to infants at birth (*7*). The vaccine, stored at room temperature, was given to 358 infants at birth by village midwives. As a control the same vaccine, stored in a refrigerator, was administered to 232 infants within 24 to 72 hours after birth by village doctors. The second and third doses were given with other vaccines as part of the mobile outreach services, which were available at intervals of about two months. The rates of seroconversion to anti-HBsAg for vaccine stored without and with refrigeration were 81.6% and 81.9% respectively.

Although HB vaccine is extremely heat stable, there are not yet enough data to recommend using it entirely outside the cold chain. The promising report from China does not include data on the range of ambient temperatures at which HB vaccine was stored and used by village midwives. There is, however, scope for developing a management instruction that would allow removal of the vaccine from the cold chain in emergencies, or in outreach activities of short duration, provided that a high-temperature indicator was attached to each vial.

5.3 Summary

These data suggest that HB vaccine is in the upper range of heat stability, together with tetanus and diphtheria toxoids, among the vaccines commonly used in the immunization programmes. The vaccine is stable for up to four years at temperatures of 2 to 8°C, for months at 20°C to 25°C, for weeks at 37°C and for days at 45°C. As with other vaccines adsorbed on aluminium salts, freezing of HB vaccine may cause a significant reduction of potency. The freezing point of HB vaccine is about -0.5°C. The vaccine should always be protected from being frozen, especially at the end of the cold chain when it is transported in cold boxes and may come into close contact with cold packs.

6. Measles vaccine

6.1 Stability of freeze-dried vaccine

In recent years significant progress has been made in improving the heat stability of measles vaccine. The development of an effective stabilizer (*59, 102, 119*) and the formulation of a WHO requirement for heat stability of freeze-dried measles vaccine (*152, 153*) have made a considerable impact on the quality of measles vaccines on the market.

This requirement uses two indices of stability:

(1) Freeze-dried vaccine should retain at least 1000 live virus particles in each human dose at the end of incubation at 37°C for seven days; and

(2) If, during incubation, the virus titre has been decreased, then it shall have done so by not more than 1 $\log_{10}$ (*152*).

The increased heat-stability under normal working conditions is especially important in the developing world (*65*).

Measles vaccine in its dried form is extremely stable in temperatures below zero (*102*). The dried vaccine stays potent if kept cold and it is not damaged by freezing and refreezing.

The thermal degradation of the second generation measles vaccines is slow (Figure 3) (*4, 100*). At 2 to 8°C these improved vaccines maintain minimum potency for more than two years (*19, 102, 119).*

The stability of present measles vaccines is pronounced at higher temperatures. At room temperature (20°C to 25°C) modern measles vaccines show low degradation rates, of about 0.17 and 0.33 $CCID_{50}$ loss respectively after two and four weeks' storage (*119*). The minimum required infectivity titre is still retained after two to four months' storage at room temperatures (*73, 102*). Measles vaccine kept at 25°C for seven days induced seroconversion in 122 (92%) of 132 children vaccinated (*66*).

Measles vaccine has been shown to maintain the required infectivity titre after 14 days storage at 37°C (*102, 119*). The second generation vaccines lost 0.39 and 0.47 $\log_{10}$ $CCID_{50}$ respectively after 7 and 14 days' storage at 37°C, still showing the residual virus concentration above 1000 $CCID_{50}$ per dose (*73*). During the whole 30-day exposure to 37°C, the degradation rate was 0.025 $\log_{10}$ $CCID_{50}$ per day. The half life was about seven days.

The enhanced thermostability of the second-generation measles vaccines has been confirmed in the field. Studies in Cameroon showed that two second-generation measles vaccines stored at 37°C for up to 14 days, were able to induce seroconversion in seronegative children (*65*).

At 41°C, the degradation of measles vaccine proceeds quickly with the titre decreasing by 50% within two days and by a 0.4 to 0.7 $\log_{10}$ loss within one week (*48, 119*). The half life of measles vaccines was estimated to be 31 days, 16.6 days and 3.3 days at 20°C to 25°C, 37°C and 41°C, respectively (*5*).

At 54 to 56°C measles vaccine is inactivated rapidly, losing more than 0.65 $\log_{10}$ and 1.3 $\log_{10}$, respectively, during one-day and three-day exposures. The time required for reduction in titre to 1000 $CCID_{50}$ was about 12 hours (*102*).

6.2 Stability of reconstituted vaccine

Measles vaccines, even those with enhanced thermostability in dry form, quickly lose their potency when reconstituted and kept at elevated temperatures.

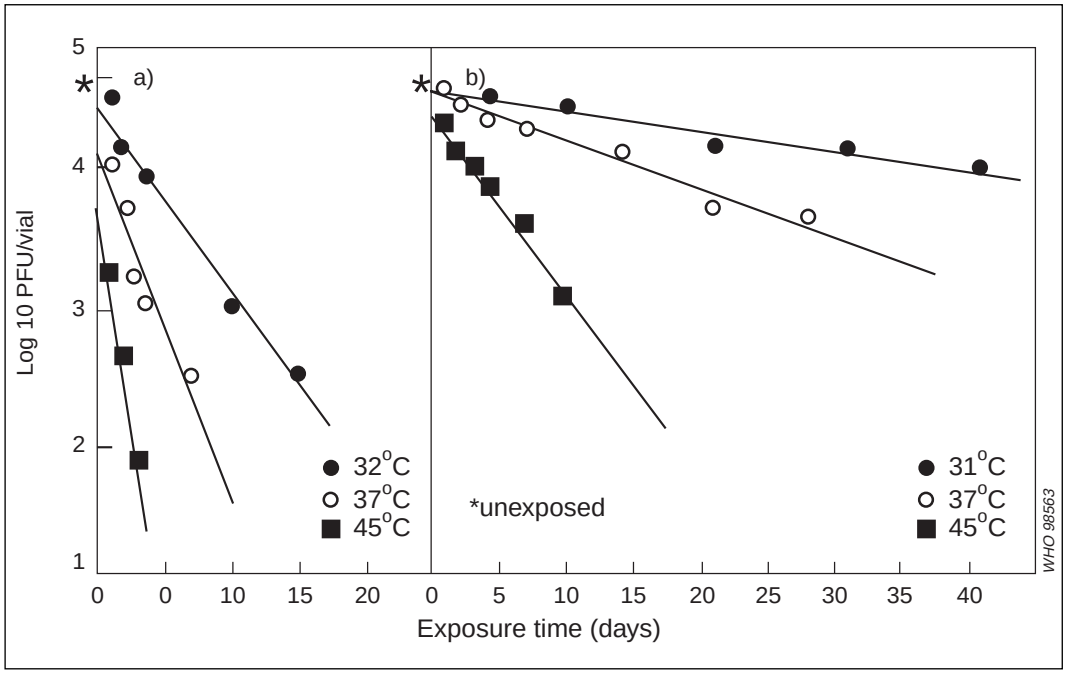
The potency of reconstituted measles vaccines at 4°C remained above 1000 $CCID_{50}$ for at least 24 hours, although the vaccines underwent degradation at a rate of 0.014 $\log_{10}$ $CCID_{50}$ per hour (*48*). When reconstituted vaccine is kept at 26°C, the titre loss per hour may reach 0.02 to 0.027 $\log_{10}$. The time required for reduction in titre to 1000 $CCID_{50}$ ranged between 16 and 24 hours (*48, 102, 73*).

At 37°C, the vaccine degradation is quicker: the titre loss per hour reaches about 0.1 $\log_{10}$ or more, so the time required for reduction in titre to 1000 $CCID_{50}$ may be shorter than seven hours (*48, 102, 119*).

Reconstituting vaccine with a warm diluent may be harmful; vaccine reconstituted with the diluent prewarmed to 41°C and then further incubated in the waterbath at that temperature lost half of its original potency after half an hour and 0.5 to 0.7 $\log_{10}$ after one hour (*119*). At 37°C the loss of titre was 0.4 to 0.5 and 0.8 to 1.0 $\log_{10}$ after three and six hours respectively (*48, 119*).

Reconstituted measles vaccine must be used in the same immunization session. Measles vaccine is produced in lyophilized (freeze-dried) form and must be reconstituted before use with diluent provided by the manufacturer. This creates an opportunity for errors to be made in handling of the vaccine (*156*). There is a serious risk when reconstituted measles vaccine is stored at any temperature for longer than six hours or above 8°C for any period. This is because of the possibility of contamination of the product, which could cause serious adverse consequences in those being vaccinated. When used, measles vaccine should be protected from elevated temperature and from light (light may inactivate the virus). Reconstituted vaccines must be discarded at the end of each immunization session and should NEVER be kept for use in subsequent sessions (*156*).

Figure 3: Accelerated stability tests at three temperatures for (a) a first-generation vaccine and (b) a second-generation vaccine



* Value for unexposed vaccines

*Sources: Allison LMC et al., and Mann GF et al. (*4, 100*)*

6.3 Summary

Measles vaccine in lyophilized form is quite stable. It is stable in temperatures below zero and it is not damaged by freezing and refreezing. At between 2°C and 8°C dried measles vaccine maintains minimum potency for more than two years. At room temperature (20°C to 25°C) the minimum required infectivity titre of measles virus is still retained for at least one month and it can be maintained for at least one week at 37°C. At 41°C measles vaccine degrades quickly with the titre decreasing by 50% within two days.

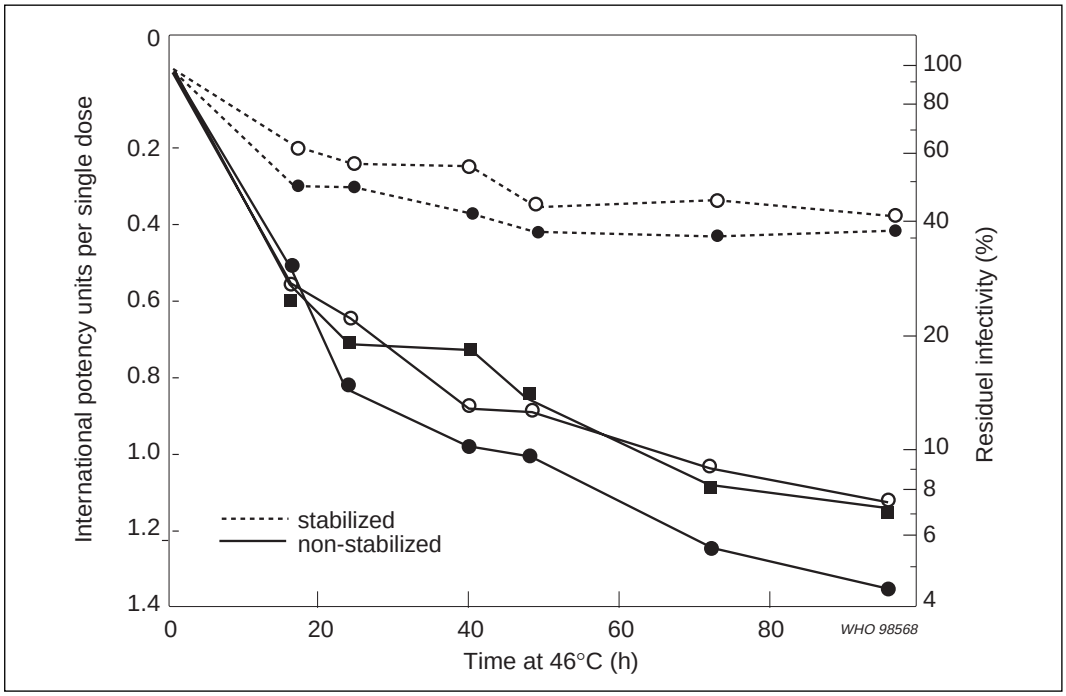
After reconstitution, measles vaccine rapidly loses its potency when kept at elevated temperatures. At a temperature of 26°C the vaccine reduces in titre to the minimum level in about 16 hours and at 37°C this takes about seven hours. Reconstituted measles vaccine should be kept cold during immunization procedures, must be discarded at the end of each immunization session and must never be kept for use in subsequent sessions.

7. Yellow fever vaccine

7.1 Stability of freeze-dried vaccine

The poor stability of the early yellow fever (YF) vaccines has been a matter of concern. Most of them lose some activity during storage at -20°C or +5°C for six months, and they deteriorate quite quickly at higher temperatures (*125, 133*). Several manufacturers have introduced vaccines with enhanced stability. Media such as lactose, sorbitol, histidine and alanine have considerably improved the heat stability of lyophilized 17D vaccine (*12, 13, 133*). Stabilized vaccines may successfully be used in different field conditions (*55, 126*). The differences in stability between the old and new YF vaccine formulations are shown in Figure 4.

Figure 4: Decrease of infectivity of lyophilized, stabilized and non-stabilized yellow fever virus vaccines kept at 46°C for four days



Source: Burfoot C, Yound PA and Finter NB (25).

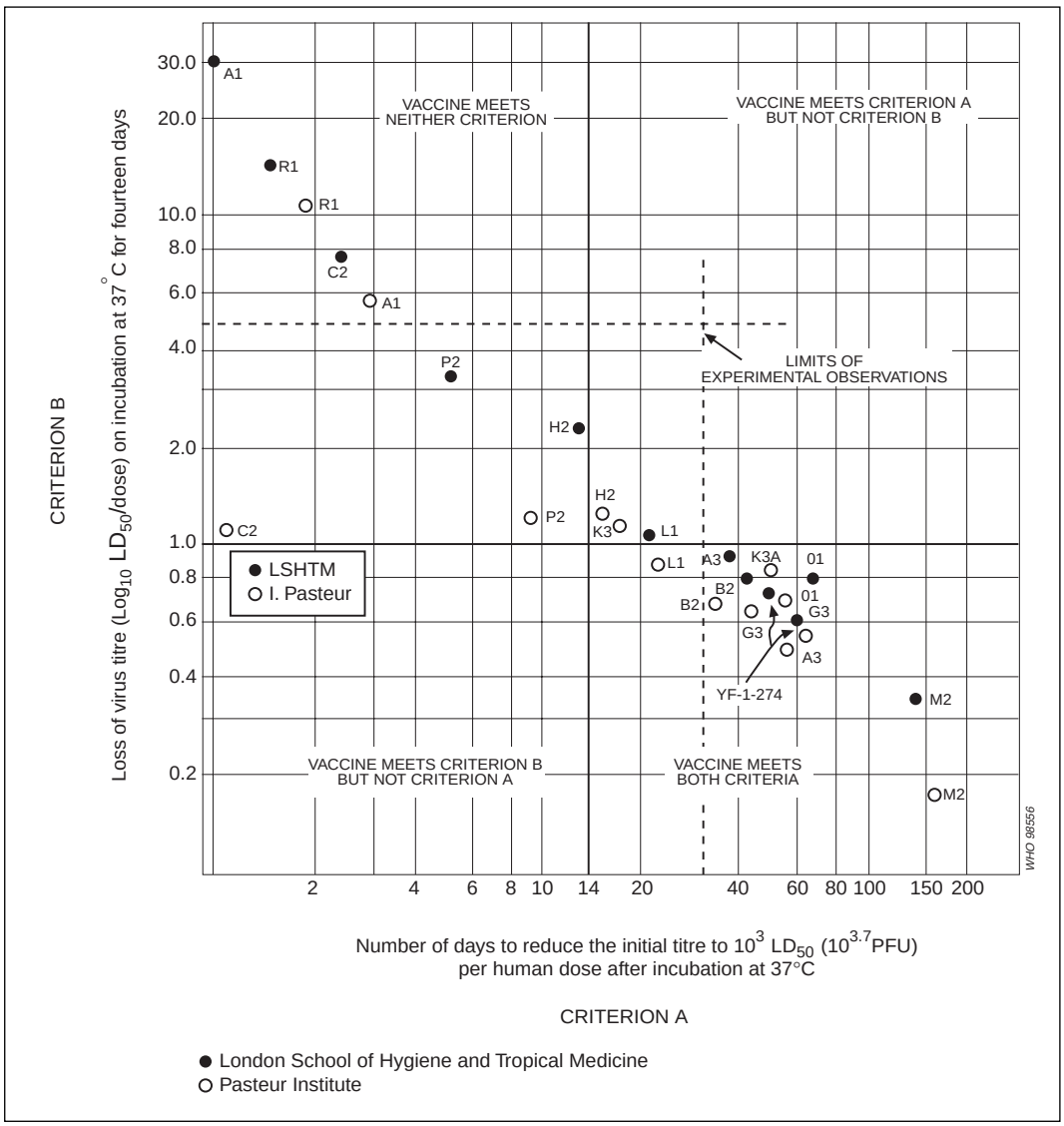
Yellow fever vaccine can safely be stored at -20°C or +4°C for two years or more (*25*). The estimated half life of the vaccine infectivity is 3 to 10 months at room temperature, from 10 to 20 days at 37°C, and about two days at 46°C (*25, 52*). The time required to reduce the initial titre of the vaccine to 1000 infective units is between 2.6 to 6.1 days at 46°C, 5.7 to 15.7 days at 37°C and 12.4 to 26 days at 31°C (*72*).

There are similar declines in titre when the stabilized vaccine is held continuously at 37°C and when it is subjected alternately to 37°C and 4°C (*52*).

In 1987, a study of 11 YF vaccines was sent to WHO by manufacturers. This showed a wide range of stability among vaccines (*148*). The number of days required to reduce the initial titre to 1000 infective units, when vaccines were kept at 37°C, ranged from one to five days in four vaccines, from 13 to 21 days in two vaccines, and from 38 to 146 days in five vaccines (Figure 5).

In Figure 5, the vaccines which appear above the horizontal line indicating the limit for a loss of 1 $\log_{10}$ of virus underwent too high a titre loss . Likewise, the vaccines which appear to the left of the vertical line which indicates 14 days of incubation experienced too high a loss of potency. Vaccines which appear in the lower-right quadrant meet both criteria A and B of the WHO requirement for stability. This requirement stipulates that the vaccine should retain 1000 mouse LD_{50} or the equivalent in plaque-forming units (PFUs) per human dose (A), and that the mean titre loss should be less than 1 $\log_{10}$ after two weeks' incubation at 37°C (B) (*155*). This requirement is met by all YF vaccines produced by WHO-approved sources (*159*).

Figure 5. Rating of 17D yellow fever vaccines according to proposed requirements for heat stability



*Source: World Health Organization (*148*)*

7.2 Stability of reconstituted vaccine

When kept at between 0°C and 8°C, the reconstituted vaccine retains its minimum immunizing dose (1000 infective units) for at least 10 days (*72*). However, when the reconstituted vaccine is exposed to elevated temperatures, it quickly deteriorates. At 37°C, 31°C and 27°C, the reconstituted vaccine lost 50% of its infectivity following 1.5, 3.1 and 4.9 hour exposures (*25, 72*). The use of a diluent at 37°C results in rapid virus inactivation and total loss of activity within one hour (*134*). An exposure of reconstituted vaccine for one hour to 46°C resulted in a 0.5 $\log_{10}$ loss, and after a two-hour exposure, the loss of infectivity exceeded 1$\log_{10}$ (*25*). Of various diluents, including saline, buffer, gelatin and peptone, distilled water was the most effective in maintaining a satisfactory virus titre of vaccine for three hours after reconstitution at 37°C (*134*).

In a study in Cameroon, the seroconversion of a group of children who were immunized with YF vaccine which had been kept after reconstitution at 25°C to 30°C for one hour, two hours, and three hours, was 100%, 82% and 67%, respectively (*37*).

7.3 Summary

Lyophilized yellow fever vaccine can be safely stored at -20°C or +4°C for two years. The estimated half life of the vaccine infectivity is 3 to 10 months at room temperature (20°C to 25°C). Reduction of the initial titre of the vaccine to the minimum acceptable level takes place in two to three weeks at 31°C, in one to two weeks at 37°C and in three to six days at 46°C. As with the measles vaccine, yellow fever vaccine quickly deteriorates after reconstitution when it is exposed to elevated temperatures. At 27°C the reconstituted vaccine lost 50% of its infectivity following a five-hour exposure. The use of a diluent at 37°C results in rapid virus inactivation and total loss of activity within one hour. Yellow fever vaccine should be quickly administered after reconstitution (up to one hour). If the reconstituted vaccine is kept continuously in an ice bath, it can be used within one immunization session but must be discarded at the end of the session.

8. Pertussis vaccine

Stability studies on pertussis vaccine are hampered by the lack of a simple, inexpensive and reproducible potency test. The potency test recommended by WHO (*149*) is technically difficult and requires highly qualified staff and a large number of mice of a specific strain. The results are subject to wide biological variation. It is difficult to obtain precise data on the deterioration of the potency of vaccine exposed to elevated temperatures unless it shows marked changes.

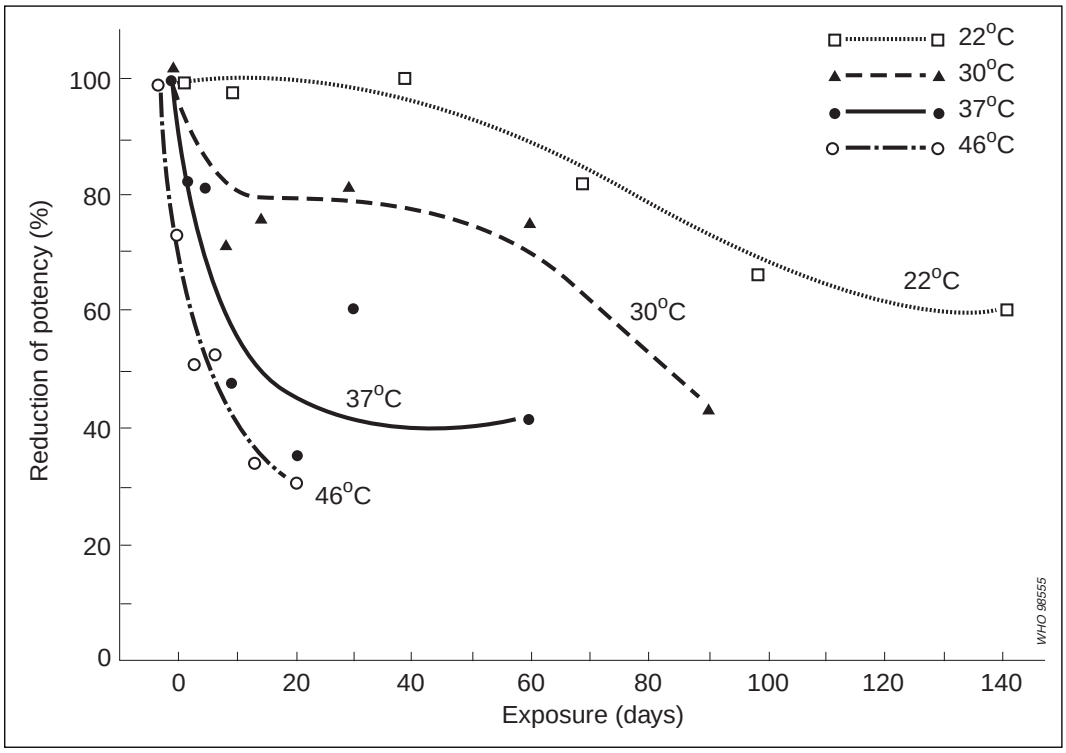
Nevertheless, several studies have provided valuable information on various factors that influence the stability of pertussis vaccine. The most frequently studied factors are:

- the temperature (*6, 34, 44, 82, 83, 85, 124, 146*);
- the form of the vaccine: monovalent vaccine versus the pertussis component of DTP vaccine (*6, 34, 69, 79*);
- the method of inactivation (*60, 77, 79*);
- the strain of *B. pertussis* (*26*);
- the nature of the adjuvant or preservative (*113, 146*).

8.1 Influence of temperature on vaccine potency and toxicity

The potency of the pertussis component of DTP vaccine depends on the storage temperature; potency may be reduced either by high temperatures or by freezing. The impact of various ambient temperatures on the potency of the pertussis component of DTP vaccine is shown in Figure 6 (*44*) and Table 4.

Figure 6: Loss in potency of pertussis component of DTP vaccine kept for various lengths of time at different temperatures



Source: World Health Organization (44)

When stored in a refrigerator between 4°C and 6°C, the pertussis component of DTP or DTP-polio vaccines appears to have satisfactory potency over a period of two years (*80, 83, 146*). However, even under optimal conditions, a continuous decrease in potency occurs during long periods of storage. DTP vaccines with an estimated average initial potency of 8.5 IU per single human dose have a potency below 4 IU per dose after 46 months (*80*). The potency of the pertussis component of DTP-polio vaccines declines from 5.2 to 8.6 IU/ml to 1.2-1.6 IU/ml during storage for three years at 4°C (*83*).

The average annual loss of potency of the pertussis component of DTP vaccines is estimated at 0.35 IU per human dose. Potency reaches a minimum of 4IU per dose after storage at 4°C for six years (*33, 34, 77*). At 22 to 25°C the potency of pertussis vaccine remains above 80% of its original value for two to eight weeks. It decreases gradually thereafter with an estimated degradation rate of 0.3% to 0.4% per day.

At 37°C, the process of vaccine degradation becomes more pronounced and seems to be biphasic: potency initially declines more rapidly, the estimated degradation rate being between 1% and 6%; later the rate of degradation decreases (*44, 60, 83, 146*).

Table 4: Stability of the pertussis component of DTP vaccines at various temperatures

Storage temperature (°C)	Reference	Estimated potency loss per day (%)	Time of storage and time used for calculation of degradation rates
4 - 8	34	0.06	6 years
	85	0	45 days
	6	0	12 - 18 months
	61	0.01	90 days, 15 - 90 days
	83	0.05 - 0.06	3 years
22 - 25	85	0.31	45 days, 0 - 45 days
	44	0.41	140 days, 40 - 140 days
	6	0	30 days followed by 18 months at 4ºC
	60	0.26	90 days, 15 - 90 days
30	44	1.80	90 days, rapid decrease
		0.80	0 - 15 days slow decrease, 30 - 90 days
35 - 37	146	3 - 6*	56 days, 0 - 7 days
	124	1.2	90 days, 0 - 15 days
	44	5.2	60 days, 0 -20 days
	60	2.4	90 days, 0 -15 days
	83	5.5	56 days, 0 - 7 days
46	82,83	6.7	56 days, 0 - 7 days
	44	10.8	20 days, 0 - 4 days

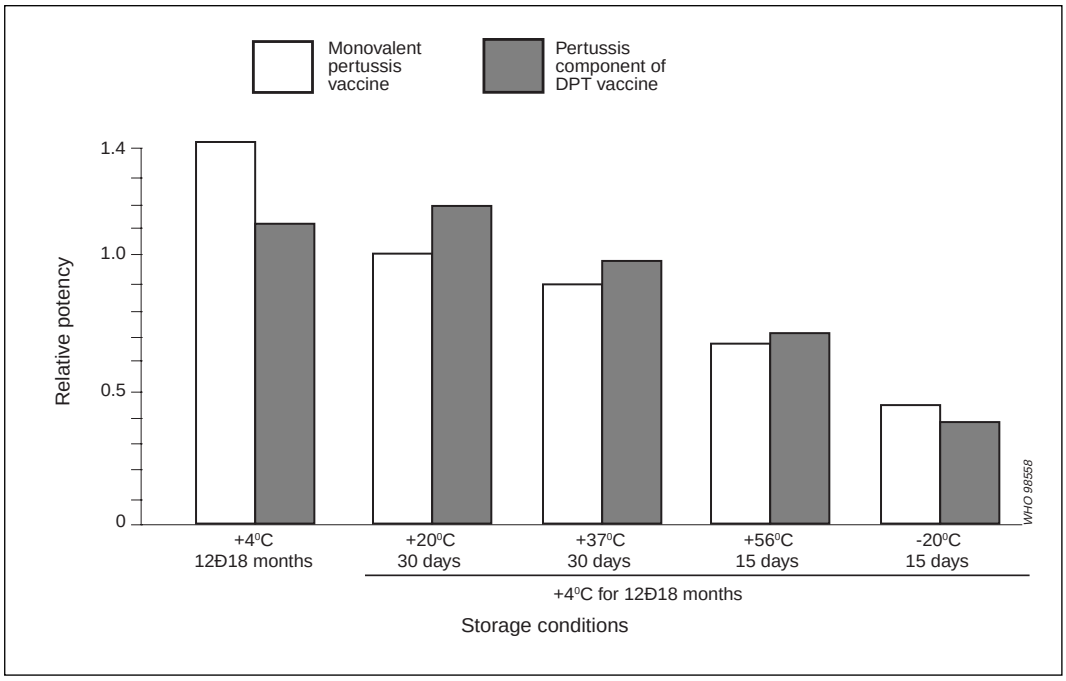
*Two DTP-polio vaccines with different preservatives

During the first few days of storage at 45°C to 46°C the decline is high, reaching some 10% per day. A loss of 50% may occur during exposure for only four to seven days (*44, 82*), and storage at 50°C to 56°C brings about rapid and complete loss of potency in the pertussis component (*15, 69*). Higher resistance of the pertussis component of DTP vaccines to elevated temperatures has been reported but not explained (*15, 85, 124, 136*).

There are no data on the stability of acellular pertussis vaccine, which, in DTP vaccine, contains proteins adsorbed on aluminium salt. A stability profile similar to that of other protein vaccines is therefore to be expected, i.e., relatively good thermostability, poor resistance to freezing and a shelf-life of two to three years at 2 to 8°C (*81*).

Freezing may impair the potency of pertussis vaccines. When DTP vaccines are submitted to freezing at -20°C for 15 days the potency of their pertussis component loses more than 50% of its initial value. The potency of the pertussis component is more impaired by freezing than by storage at elevated temperatures (Figure 7) (*6*). When adsorbed DTP vaccines from five manufacturers were kept for 12 hours at between -5°C and -10°C and between -20°C and -30°C, three of them underwent significant losses in the potency of the pertussis component in both temperature ranges (*42*).

Figure 7: Immunogenicity* of monovalent pertussis vaccine and the pertussis component of DTP vaccine stored under various conditions



* Expressed as relative potency in comparison with the national reference preparation. Relative potency 0.5 = 4 IU per single dose.

Source: Andrescu V et al. (6)

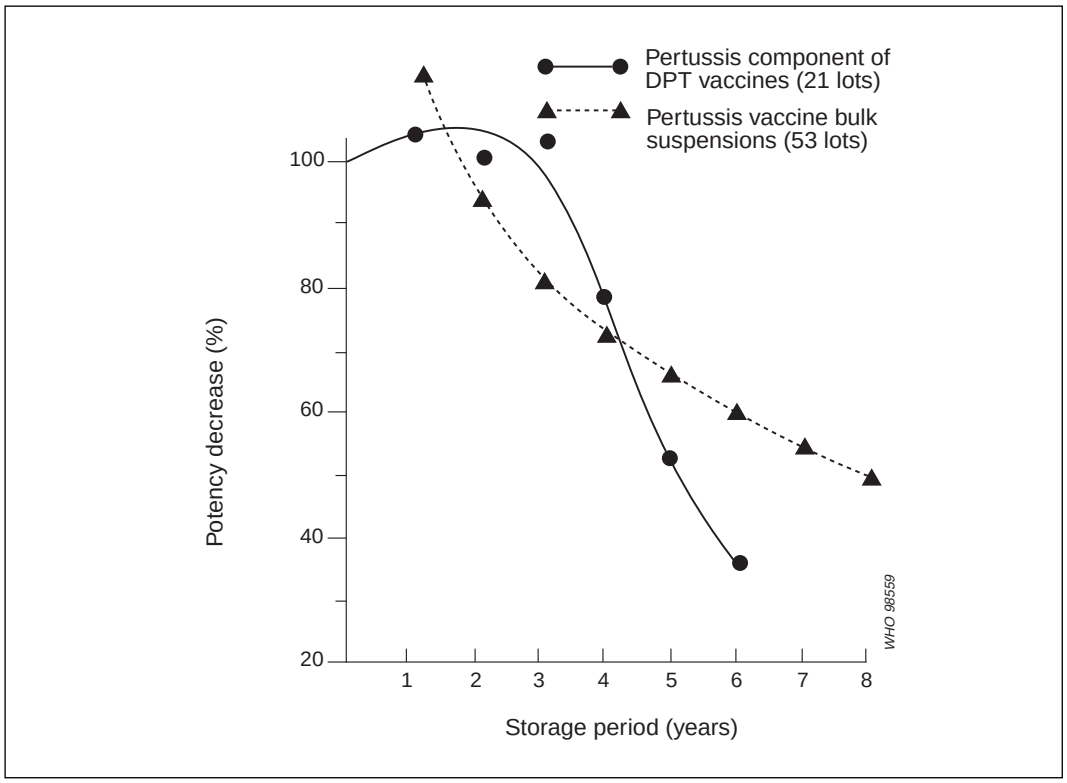
There is no evidence that the toxicity of pertussis vaccine increases with storage, as measured by the mouse weight gain and histamine-sensitizing tests (*6, 26, 61*). In fact, vaccine samples kept at 25°C and 35°C for between four weeks and three months showed reduced toxicity (*26, 61*).

8.2 Monovalent pertussis vaccines versus the pertussis component of combined vaccines

In one study, monovalent pertussis vaccines were evidently unstable at 4°C: during storage for 18 months some samples lost 58% to 87% of their initial potency (*69*).

During the first year of storage, *B. pertussis* bulk suspensions seem to deteriorate more rapidly at 4°C than the pertussis component of DTP vaccines adsorbed on aluminum phosphate (Figure 8), probably because they lack the protective effect of the toxoid proteins and the aluminum ions in the triple vaccine. (The influence of aluminum ions is discussed in section 8.5.)

Figure 8: Potency of *B. pertussis* bulk suspensions and the pertussis component of DTP vaccines stored for one to eight years at 4°C



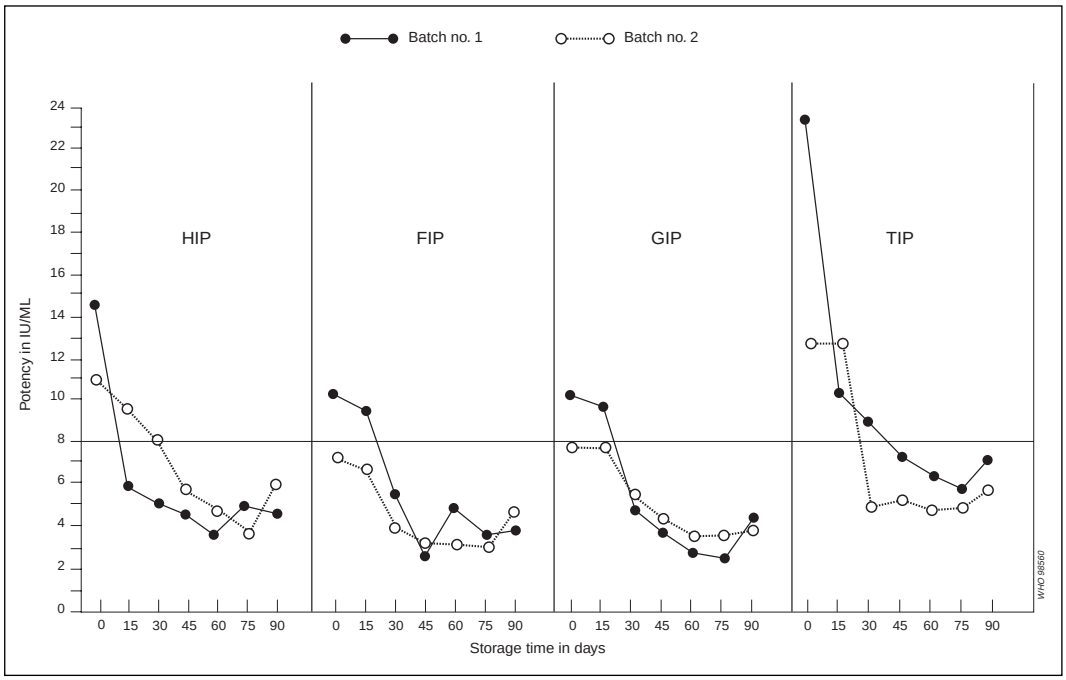
*Sources: Csizer Z, Zsidai J, Joo I (*33, 34*).*

8.3 Methods of inactivating B. pertussis

Early studies on the stability of pertussis vaccines prepared from cultures grown and killed by various methods suggest that, of the inactivating agents merthiolate, phenol, formalin and heat, none stands out as definitely superior. During prolonged storage, however, vaccines killed with phenol or formalin become dark in colour and difficult to resuspend, while those killed by merthiolate or heat show little change in appearance.

Early observations of Kendrick (*79*) were confirmed by Gupta et al., who studied the stability of DTP pertussis components prepared with different methods of inactivation (heat, formaldehyde, glutaraldehyde, thiomersal or acetone treatment) (Figure 9) (*60*). Stability tests performed after the storage of vaccines at 4°C to 8°C, 25°C and 35°C for 90 days showed no differences in stability attributable to the inactivating agents used.

Figure 9: Potency of pertussis component of DTP vaccines stored at 35°C following inactivation by heat (HIP), formaldehyde (FIP), glutaraldehyde (GIP) and thiomersal (TIP)



*Source: Gupta RK et al. (*60*)*

The work of Gupta et al., demonstrates the problems encountered in studies on pertussis vaccines: low reproducibility in vaccine potency estimates and differences in degradation rates of vaccines prepared in the same way. The initial potency of vaccines prepared by different inactivation methods differs considerably, with thiomersal-inactivated vaccines having the highest potency and acetone-treated vaccines being of substandard potency.

8.4 Strains of B. pertussis

In research (*26*), vaccines prepared from six different *B. pertussis* strains showed some variation in stability but the numbers were too small to make a clear distinction between stable and unstable strains. A strain that led to a vaccine of higher stability, however, retained both leukocytosis-promoting factor activities and histamine-sensitizing factor activities (measures of pertussis toxin) longer than vaccines prepared from other strains.

8.5 Influence of preservative and adjuvant

A significant loss of potency may occur in the pertussis vaccine component of DTP-polio vaccine if the preservative is benzethonium chloride (BC) (*38, 122*). BC was introduced as a preservative to replace merthiolate, which was shown to inactivate the poliovirus component. It is a quaternary ammonium compound whose probable mode of action involves attachment to the negatively charged sites on the cell surface of *B. pertussis*.

Subsequently, Olson et al., showed that pertussis vaccine preserved with BC at 37°C for 16 weeks had no measurable mouse-protective potency (Table 5) (*113*). However, the impairing effect of BC treatment was reduced by treating the vaccine with aluminum, calcium or magnesium salts or with choline or D-lysine before the addition of the preservative. It was suggested that these substances prevented the absorption of BC on to the pertussis cells, thus stabilizing the protective antigens as measured by the mouse protection test.

Van Ramshorst and van Wezel (*146*) studied the stability of all components of quadruple DTP-polio vaccines preserved with BC, 2-phenoxyethanol and formaldehyde. The rates of potency loss in the pertussis component of vaccines preserved with BC and 2-phenoxyethanol were not essentially different from the rate in vaccine preserved with merthiolate. It is possible that the deleterious effect of BC was reduced by the aluminium phosphate in the vaccines.

Table 5: Potency of pertussis vaccines containing various preservatives and stored at 37°C

Preservative	Potency in IU/ml Length of storage at 37°C				
	0 weeks	5 weeks	10 weeks	16 weeks	42 weeks
Merthiolate	4.6	2.1	2.1	-	NP
Benzethonium chloride (BC)	4.7	2.8	0.8	NP	NP
BC + calcium chloride	3.6	3.6	3.0	3.6	3.3
BC + aluminum phosphate	4.3	8.5	3.8	-	3.4
BC + magnesium sulfate	7.1	2.8	2.8	1.9	0.9
Choline	-	4.4	2.3	-	2.2

NP = no protection found.

*Source: Olson BH, Eldering G, Graham B (*113*).*

8.6 Summary

In its usual presentation, DTP with thiomersal and aluminum adjuvant is susceptible to freezing but relatively stable at 4°C for two years or more. It is resistant to storage for several months at 22 to 25°C, for several weeks at 37°C, and for less than one week at 45°C. As with most protein-containing vaccines, temperatures higher than 56°C are immediately deleterious.

9. BCG vaccine

The standardization of the stability of BCG vaccine and studies on it are complicated by the following factors:

(1) Different substrains of BCG at various levels of attenuation are used in vaccine production.

(2) There are differences in the manufacturing and testing procedures employed by vaccine producers. The technique and time of cultivating BCG and the nature of the stabilizer are important factors.

(3) There are differences between products in bacterial content and the number of culturable particles (CPs).

(4) There is no approved laboratory method for assaying the protective potency of vaccines against tuberculous infection in humans.

The most important element in batch-to-batch quality control is the checking of vaccine viability. This involves determining the number of CPs by means of colony counts on solid medium. The viability test is also of prime importance in assessing the stability of BCG stored in different conditions.

BCG vaccine was the first vaccine for which a WHO requirement for heat stability was established (*150*). An ADT should be conducted on each lot of BCG vaccine. The number of CPs in vaccine incubated at 37°C for 28 days should be not less than 20% of that in the vaccine stored at 4°C (*154*).

9.1 Impact of temperature on the viability of BCG vaccine

BCG vaccine is relatively stable at refrigerator temperatures below 8°C, and most manufacturers give a validity period of one year if this condition is met. Viability decreased by about 20% during storage at 4°C for two years (*53*), suggesting an annual loss of about 10% (*53, 165*) below 8°C. However, some vaccines can lose 20% to 25% of their original viability during storage for only six months (*137*).

BCG vaccines showed only a slight loss of viability when kept at 13–15°C for two months, but the decline reached about 20% at the end of nine months (*24*). Exposure to 18°C reduced viability by about 10% a month (*165*).

At room temperatures (in the range 20°C to 25°C) some BCG vaccines may lose 25% to 40% of their original viability during storage for two months (74) and one-fifth to one-third of their viability in three months (*24*). At 30°C or 37°C, degradation begins very rapidly and the rate of CP reduction is greater at the beginning than during later stages of exposure (Table 6) (*24, 129*). It is not known whether this early degradation rate would be as steep if exposure to high temperatures were repeated. The daily loss of viability in vaccines kept at 37°C for a few weeks ranged from 1% to 2% (*11, 20, 24, 74, 165*).

Table 6: Loss of viability in four BCG vaccines stored at 30°C and 37°C for 36 weeks

	Rates of potency loss per day (% of original values) at			
	30°C		37°C	
	Storage period (weeks)			
Vaccine	**0 - 9**	**10 - 36**	**0 - 4**	**6 - 36**
Japanese	0.5	0.1	0.8	0.2
Glaxo	0.9	0.2	2.1	0.1
Dakar	1.0	0.2	2.3	0.1
Danish	0.8	0.2	1.9	0.2

Source: Bunch Christensen K (24).

At temperatures above 37°C the degradation of BCG vaccine is very rapid. Exposure to 54°C results in the loss of 25% to 73% of the original viability in one day and of 74% to 85% in three days (*74*). Viability may be reduced by half during exposure to 70°C for 30 minutes or by 80% during boiling for five minutes (*56*).

The reduction in the number of CPs caused by exposure of BCG vaccine to elevated temperatures is proportional to the temperature and the duration of exposure. However, since the optimal dose of the vaccine is not known, it is difficult to determine a permissible limit of heat degradation. When schoolchildren were given vaccines, the viability of which had been reduced to 40 to 60% during exposure to high temperatures for two to four weeks, the resulting tuberculin sensitivity and vaccination lesions were indistinguishable from those produced by control vaccines that had been stored in a refrigerator (*20, 165*). Longer storage at elevated temperatures reduced post-vaccination allergy and the size of vaccination lesions (*20*). The interpretation of these results is not easy, since delayed hypersensitivity to tuberculin and local vaccination granulomas, the hallmarks of specific cellular responses, are not directly related to protection.

Questions are often raised about the advisability of storing BCG vaccine below 0°C. Furthermore, it has been suggested that repeated freezing and thawing could have a deleterious effect. However, the experimental evidence indicates that viability is unaffected by storage at -20°C or -30°C or by freezing and thawing up to 10 times (24, 56).

9.2 Stability of vaccines produced from different BCG substrains

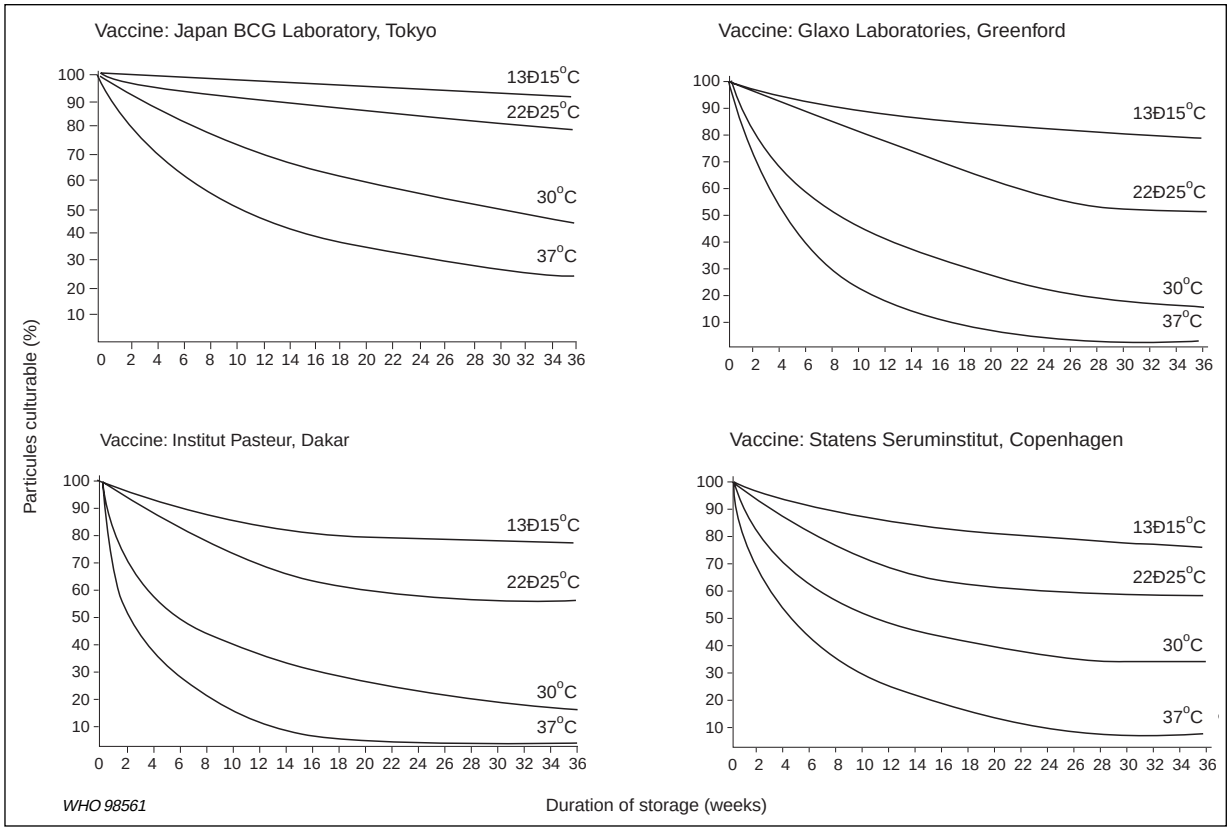
All available BCG strains are derived from the one produced by Calmette more than 65 years ago. After the long period of maintenance by culture medium transfers of the original strain there are essential differences between daughter strains.

BCG strains are usually classified either as strong, as with the French strain 1172 (Pasteur) and the Danish strain 1331 (Copenhagen), or weak, as with the Japanese strain 172, the Brazilian strain Moreau, and the British strain 1077 (Glaxo). This

distinction is based mainly on growth characteristics, residual virulence in animals and reactogenicity in children. The differences may be linked with surface antigenic lipid content and secreted protein (*1*).

There are differences in the heat stability of BCG vaccines prepared from different substrains (Table 7, Fiure 10) (*24*). At all temperatures tested, the degradation rates were lowest for the Japanese vaccine and highest for the Dakar and Glaxo vaccines, with the Danish vaccine occupying an intermediate position. During longer storage the differences between vaccines declined.

Figure 10: Viability of four BCG vaccines stored for 36 weeks at various temperatures



*Source: Bunch Christensen K (*24*).*

A recent study confirmed differences in thermostability between vaccines prepared from various substrains (*74, 75*). Japanese vaccine prepared from substrain 172 had a higher thermostability than French (strain 1172), Danish (substrain 1331 Copenhagen) and Polish (substrain Moreau) vaccines (Table 5) (*74*). At 37°C the time required for a 50% decrease in viability (CPs/mg) of Japanese vaccine was about 56 days, while for the other vaccines it ranged from 28 to 35 days. At 54°C the Japanese vaccine retained more than 50% of its viability for longer than nine days, while the other vaccines lost more than 50% of their original activities in one to three days.

Table 7: Culturable BCG particles in Japanese, Danish, French and Polish BCG vaccines at various temperatures

Temperature	Number of days	Number of culturable particles/mg			
		Vaccine			
		Japanese	Danish	French	Polish
4°C	Control	47.86	4.68	7.76	6.17
20°C	28	45.71	3.72	6.31	4.57
	63	44.67	3.02	5.89	3.72
	84	39.81	2.24	4.90	2.75
	112	38.02	2.09	3.47	2.19
37°C	14	46.77	3.80	5.50	4.68
	28	37.15	2.34	4.90	2.69
	35	-	-	-	1.62
	42	-	1.32	1.51	0.55
	56	26.92	-	1.55	-
	84	17.78	2.29	0.41	-
54°C	1	47.86	3.47	2.75	1.66
	3	46.77	1.26	1.07	0.91
	6	39.81	0.98	0.63	0.25
	7	-	0.20	0.22	-
	9	32.36	-	-	-

*Source: Janaszek W (*74*).*

Other studies have also shown differences in stability between BCG vaccines (*57, 129* – see also Table 8).

Table 8: Viability and heat stability of ten BCG vaccines

Vaccine	Initial number of CPs (x 10^6/ml)	Viability after storage for 28 days at 37°C		Daily loss of viability (%)
		CPs (x 10^6/ml)	% of initial number of CPs	(storage period analysed, days)
Japanese	27.0	16.6	61	(0 - 28)
Glaxo	20.1	10.9	54	(0 - 21)
USSR	7.1	3.6	51	1.7 (0 - 28)
Connaught	6.9	0.2	3	6.7 (0 - 14)
Dakar	6.5	1.8	28	3.2 (0 - 21)
Bilthoven	4.2	1.3	31	4.9 (0 - 14)
Copenhagen	2.9	1.9	66	2.5 (0 - 28)
Merieux	2.8	0.3	11	3.3 (0 - 28)
Pasteur Inst.	2.7	1.3	48	1.9 (0 - 28)
Prague	1.1	0.2	18	5.2 (0 - 21)

*Source: Lugosi L (*92*).*

9.3 Packing BCG vaccines

BCG vaccines require special precautions to ensure sufficient stability. In this connection the most important measures are lyophilization, the use of an effective stabilizer, and proper sealing of vaccine containers.

Increased stability at 4°C and 37°C and higher starting viability values (i.e., better survival rates after freeze-drying) have been observed after changing the composition of the stabilizer and improving the drying method (*53*).

At present the use of ampoules sealed under vacuum is the most common practice for increasing stability. However, vacuum-sealing is difficult compared to sealing in the presence of inert gas. There were no significant differences between BCG vaccines sealed under vacuum and under nitrogen or carbon dioxide at either 4°C or 37°C (*53, 86*). Viable counts for vaccine sealed under nitrogen have been reported to decline more rapidly than those for vaccine sealed under vacuum (*20*). A BCG vaccine sealed under argon seemed to have less stability at 37°C than vaccine sealed under vacuum (*56*).

BCG vaccines in rubber-stoppered vials have a lower stability than those conserved in ampoules (*92, 129*). A further disadvantage of rubber-stoppered vials is that users are tempted to keep reconstituted vaccine (*141*).

9.4 Effect of light on the stability of BCG vaccine

Freeze-dried BCG vaccines, regardless of their substrain, are sensitive to ultraviolet and fluorescent light. They should be packed in ampoules made from a substance of low light transmittance, such as amber glass, and should be protected from light when used (*87*).

9.5 Stability of reconstituted vaccine

Reconstituted BCG vaccine is very unstable and should be used during one working session of five to six hours. Residual vaccine should be discarded at the end of the session. The reasons for these precautions are as follows:

(1) There is a risk of contamination because BCG vaccine, in contrast to all other vaccines, does not contain any bacteriostatic agent.

(2) There is a loss of potency (*41*).

9.6 Summary

Most freeze-dried BCG vaccines are stable at temperatures of 0°C to 8°C.

At room temperature stability varies; after storage for several months a loss of viability of approximately 30% can be expected. The daily loss of viability in vaccines kept for a few weeks at a temperature of 37°C ranges between 1% and 2%. Reconstituted vaccine is very unstable. Once reconstituted, all BCG vaccines should be discarded at the end of one session, regardless of how many doses remain in the vial or ampoule.

10. Poliomyelitis vaccine

Remarkable progress has been achieved over the past years towards global eradication of poliomyelitis. During 1996, more than 400 million children were immunized during national immunization days, and for 1997, 500 million children were targeted. The oral poliomyelitis vaccine (OPV) has been the vaccine of choice for this campaign.

Oral poliomyelitis vaccine is the least stable of the vaccines commonly used in national immunization programmes. It uses a living, attenuated virus, which, similarly to most viruses, is unstable except when held at very low temperatures (frozen). Current recommendations require that, for maintenance of potency, the vaccine must be stored and shipped at low temperatures. The vaccine's thermostablity is being improved through the use of stabilizers such as high concentrations of magnesium chloride and some sugars. These are systematically used to stabilize all OPV preparations.

10.1 Overall stability of poliomyelitis vaccine at elevated temperatures

The stability of trivalent poliomyelitis vaccine has usually been monitored by measuring the total content of live viruses of three serotypes (*22, 46, 132*). This practice may overlook changes in the type 2 and type 3 content following exposure to elevated temperatures (*10*). Little loss in virus titre has been observed after long-term storage of OPV at -20°C (*52, 132*). Most manufacturers indicate that their OPV vaccines are potent if stored at -20°C or less until the expiry date indicated on the packing

(usually two years). When distribution is not imminent, it is advisable to store the vaccine at temperatures of -20°C or less, since this halts deterioration in vaccine potency.

The loss of potency at refrigerator temperature may vary. Peetermans and Colinet showed a loss in titre in their $MgCl_2$ stabilized vaccine of only 0.3 $\log_{10}$ after incubation at 4°C for 18 months (*117*). However, other results suggest a lower stability at this temperature range; the observed results indicated a loss of 0.15-0.31 $\log_{10}$ after a shorter (30-day) period of incubation at 5°C (*157*).

The degradation rate is proportional to the exposure temperature. Oral poliomyelitis vaccines may lose 4% to 13% of their activity per day at 25°C, 11% to 21% per day at 31°C, and 26% to 34% per day at 37°C (*157*). Half lives of different OPV vaccines tested in India were 4.3 days at 22°C and 1.7 days at 36°C (*132*). The average titre loss per day was 0.03-0.04 $\log_{10}$ $CCID_{50}$ when vaccines were kept at 26°C, and 0.10-0.12 $\log_{10}$ at 37°C (*48*). With these degradation rates a vaccine with a total virus content of 6.15 $\log_{10}$ $CCID_{50}$ loses half of its potency during a two to three-day exposure to 37°C or a seven to ten- day exposure to 22-26°C. This corresponds well with the previous observations of Melnick and Wallis (*105*) and Perkins and Magrath (*121*), who considered that poliomyelitis vaccines retained minimum potency for three days at 37°C and for 14 to 21 days at 25-28°C.

At temperatures over 37°C, the degradation of poliomyelitis vaccines is rapid. At 41°C, they lose about half of their potency daily (*48*), while at 50°C a vaccine loses 0.1 $\log_{10}$ $CCID_{50}$ in one hour (*22*), equivalent to a half life of three hours.

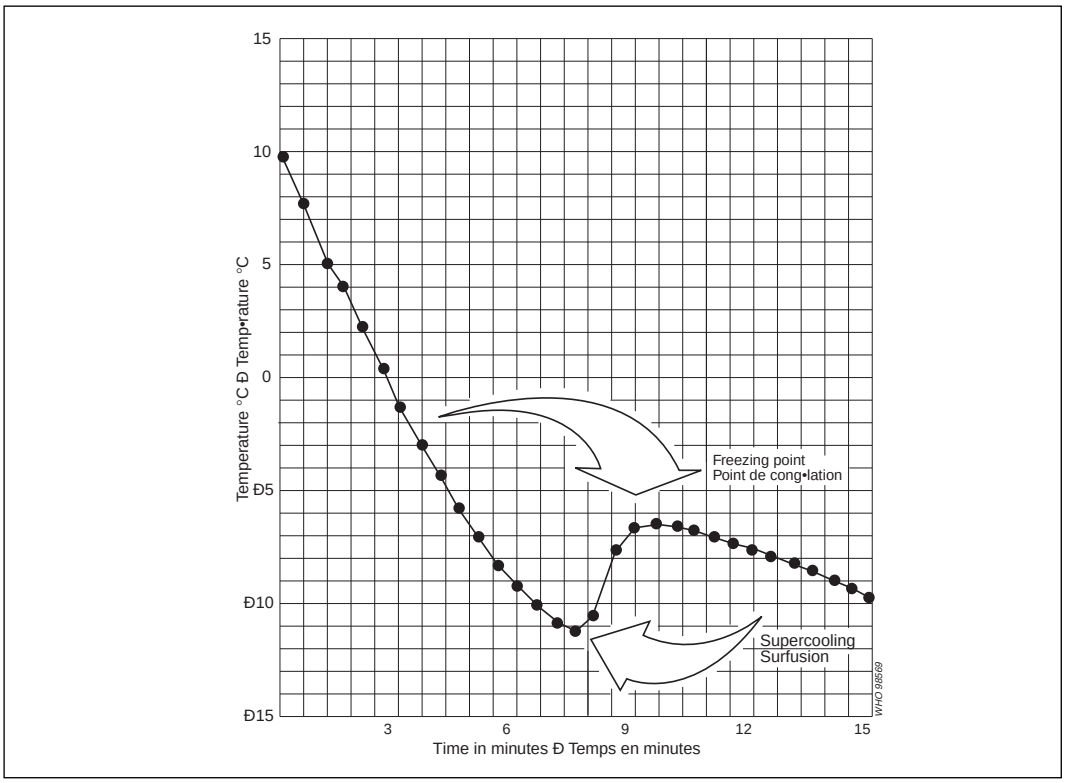
10.2 OPV at freezing temperatures

10.2.1 Freezing point of OPV

The presence of stabilizers in vaccine preparations lowers their freezing point. A study was carried out on trivalent OPV from five manufacturers to determine the freezing points of the products and the effects on their potency of up to 180 cycles of freezing and thawing (*49*).

When poliomyelitis vaccines are kept at -25°C they supercool rapidly to between -8°C and -16°C, while remaining in the liquid state. Their temperature then rises rapidly to about -7.5°C while solid freezing occurs. The temperature to which the vaccine rises is taken as the freezing point (Figure 11), which varies from -6.6°C to -8.1°C (*49*).

Figure 11: Temperature of trivalent poliomyelitis vaccines exposed to -25°C for 15 minutes



*Source: World Health Organization (*49*)*

10.2.2 Vaccine potency after repeated freezing-thawing cycles

Some studies have shown that there is no significant loss of virus titre in OPV subjected to up to 10 cycles of freezing and thawing (*18*, *40*, *46*, *52*, *132*). However, no details were given concerning the rapidity of freezing and thawing, the temperature to which samples were raised during each thawing, or the length of the intervals during which the vaccine was kept thawed. The total titres for trivalent vaccine were measured but no data were given for type-specific poliovirus sensitivity to freeze-thaw cycles. All these factors may influence the survival of virus particles during such cycles (*10*).

Vaccines subjected to 10, 90, and 180 freeze-thaw cycles (from -25°C to 2.5°C) had virus titre values for all three types which were not significantly different from those of control samples held at -25°C. There was no trend towards a decline in titre as the number of cycles increased (*49*). However, the maximum temperature did not exceed 2.1°C. Under field conditions, a break in the cold chain can result in vaccines reaching much higher temperatures. Consequently, these results are valid only for situations where the temperature of thawed vaccine remains in the range found in a refrigerator which is working properly.

Freezer compartments of refrigerators, which are sometimes used for the storage of OPV, operate at about -5°C. This is above the melting point of the vaccine, which may not, therefore, remain solid.

10.3 Recommended storage temperatures

Since there is a close relationship between storage temperature and poliovirus survival, manufacturers recommend expiry dates for OPV according to the temperature at which it is kept. Many give two figures: (1) up to two years if the vaccine is stored in a deep freezer at or below -20°C; and (2) for six months if it is stored in a refrigerator at 0°C to 8°C. One manufacturer stated that its magnesium chloride-stabilized vaccine maintains adequate immunogenicity for 12 months when kept in a refrigerator at 2-8°C, for three weeks at 25°C, and for three days at 37°C.

Constant monitoring of the cold chain system is necessary. In a study in India, the frequency of loss of potency of OPV was comparatively high initially and decreased with time (*35*). When the distribution and administration of OPV is not imminent, storage is recommended at less than -15°C for up to eight months at central level and for up to three months at regional level, as reliable freezers are usually available in these locations.

In the field, where the chance of a serious break in the cold chain is high and freezers are less common, the WHO management recommendation is that OPV should not be kept at refrigerator temperatures (0°C to 8°C) at health centres for more than one month, nor transported at these temperatures for more than one week (*45, 91*). The freezing compartment of a refrigerator in a health centre should be reserved for ice packs.

10.4 WHO requirements for thermostability

Each final lot of OPV must undergo the accelerated degradation test to confirm that its stability is satisfactory. Representative final containers of the vaccine have to be incubated at 37°C for 48 hours. The total virus content in both exposed and unexposed vials is determined concurrently with that of a trivalent reference preparation. The vaccine passes the test if the loss on exposure is not greater than a factor of $10^{0.5}$ infectious units per human dose. The national control authorities are to specify the minimum virus titres per human dose (*158*).

10.5 Factors affecting OPV stability at high temperatures

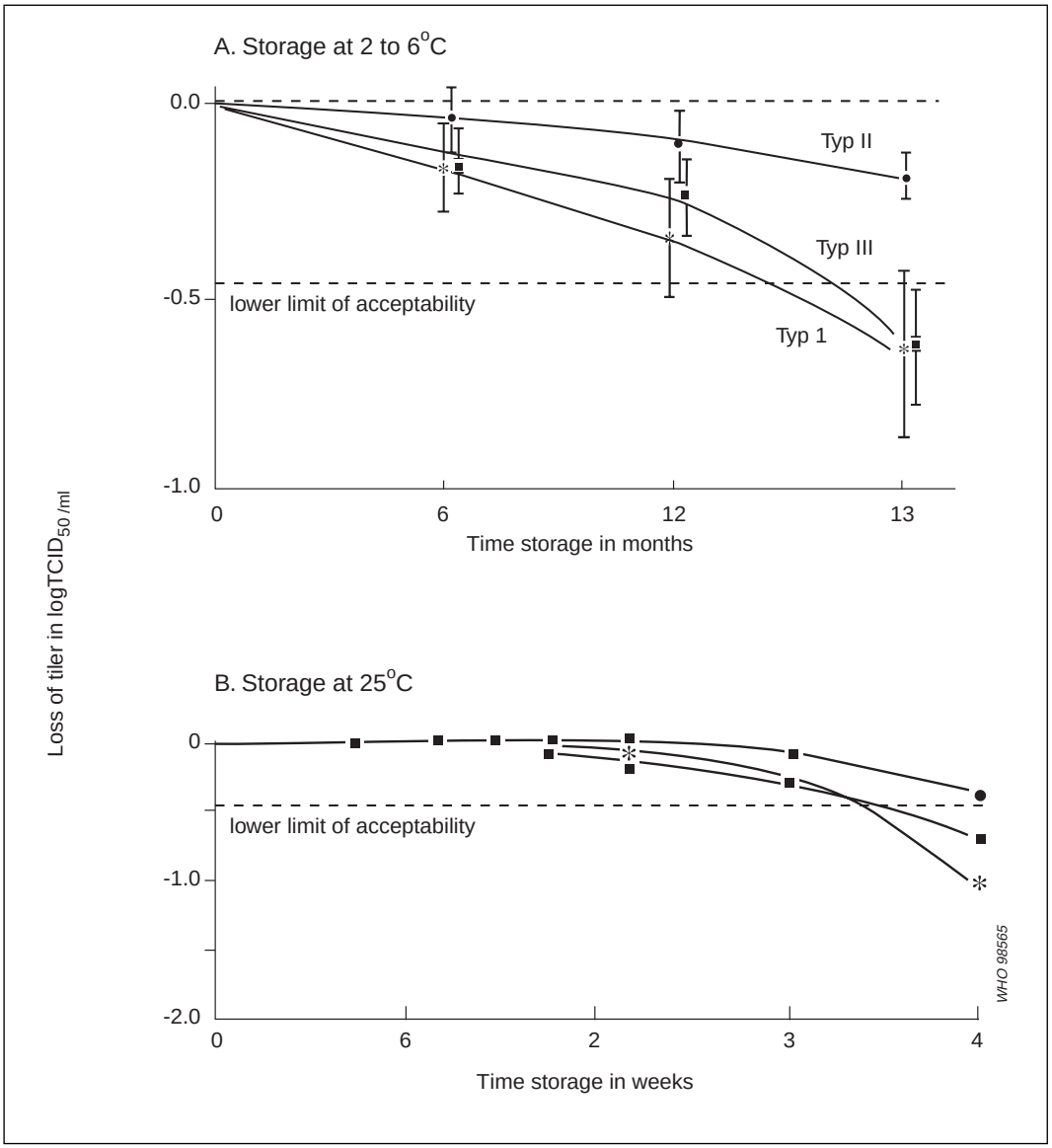
The stability of OPV depends on several factors, including possible differences in heat sensitivity between viral types, the presence and nature of a stabilizer, the pH value of the vaccine, and the container in which the vaccine is stored.

10.5.1 Differences in heat sensitivity of viral types

The individual poliovirus types in the triple vaccine differ in their growth characteristics. Type 2, when administered in conjunction with types 1 and 3, has the most prolific growth during intestinal replication, followed by types 3 and 1. To compensate for these differences in growth rate, balanced formulations of trivalent vaccine were developed, usually containing types 1, 2 and 3 in the proportions of 10:1:3 (*158*). Further studies showed that changes in the ratio of these components might enhance the immunogenicity of OPV, particularly of the type 3 virus (*114, 115*).

Tests on 50 commercial lots of OPV stored at 2 to 6°C suggested that type 2 was particularly stable and that type 1 was the least stable. The same differences in stability were found when vaccines were stored at 25°C (Figure 12) (*101*).

Figure 12: Stability of trivalent oral poliomyelitis vaccine stabilized with buffer-peptone and stored at 2 to 6°C and 25°C



*Source: Magrath DI (*96*).*

These observations have not been confirmed by other authors who tested different OPV vaccines. According to Peetermans et al. (*118*), type 1 was more stable than types 2 and 3. Type 1 showed a loss of only 0.06 $\log_{10}$ $CCID_{50}$ after storage of OPV vaccine at 4° C for 12 months, whereas types 2 and 3 showed losses of 0.20 and 0.27 $\log_{10}$ $CCID_{50}$ respectively. The differences between types were not consistent and there was no clear evidence of higher resistance of a particular type when vaccines were stored at 20°C to 25°C and 37°C (Table 9). Mirchamsy et al. could not find differences between poliovirus types kept for nine months at 4°C and -20°C (*109*).

Table 9: Comparison of stability of different poliovirus types at 20°C to 25°C and at 37°C

	Loss of titre in log_{10} $CCID_{50}$ per day at temperatures of:						
Vaccine	**20-25°C**			**37°C**			**References**
	Type 1	**Type 2**	**Type 3**	**Type 1**	**Type 2**	**Type 3**	
$MgCl_2$-stabilized	0.026	0.024	0.021	0.182	0.193	0.160	*104*
Sucrose-stabilized	0.043	0.064	0.040	0.220	0.302	0.214	
B	-	-	-	0.149	0.159	0.144	
C	-	-	-	0.151	0.129	0.136	*101*
D	-	-	-	0.207	0.171	0.150	
E	-	-	-	0.224	0.161	0.211	

10.5.2 Nature of stabilizer

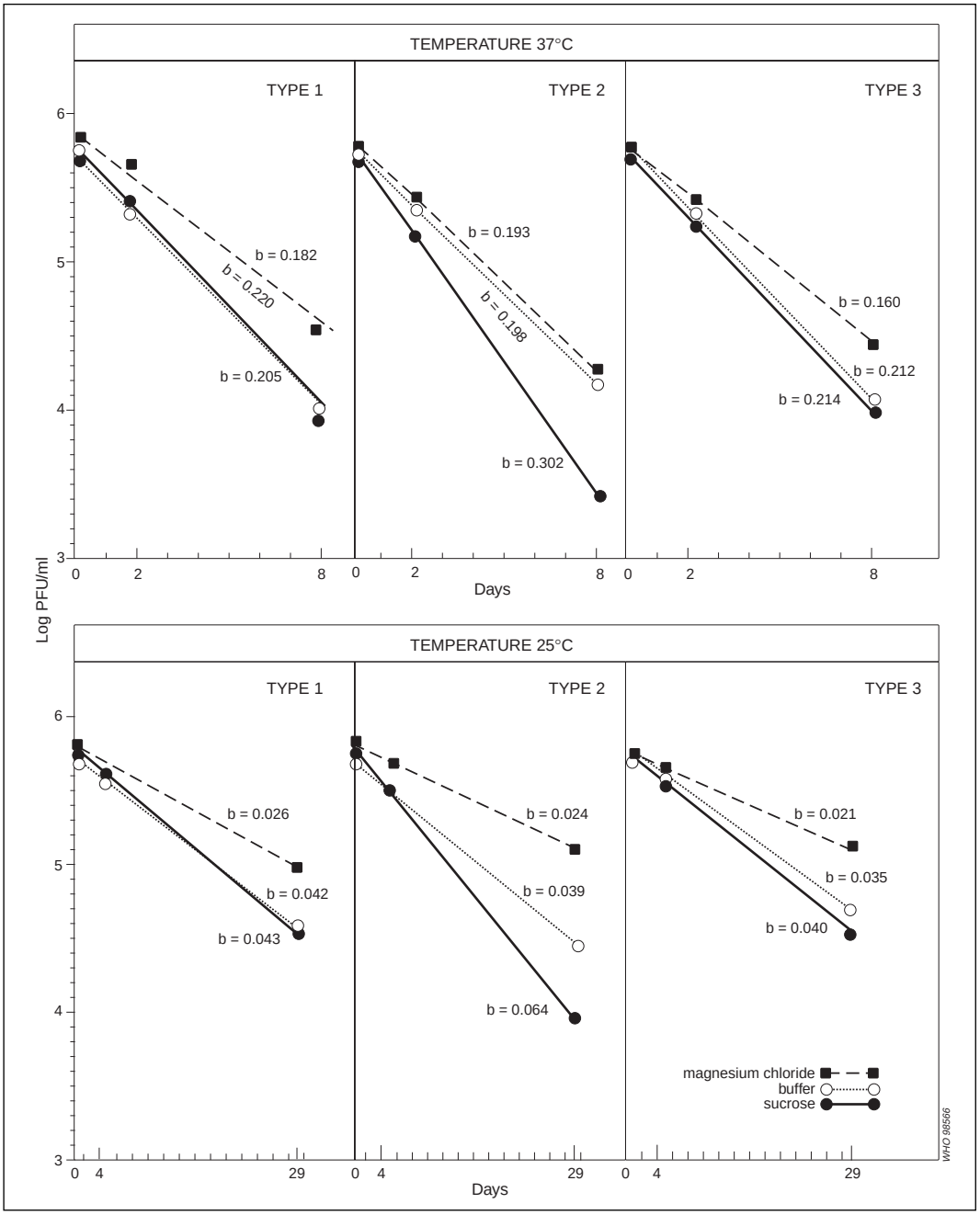
The stabilizers most frequently used with attenuated polioviruses have been magnesium chloride and saccharose; buffers, milk and gelatin have also been used.

Wallis and Melnick (*147*) reported that polioviruses were stabilized by the addition of cations to the suspending medium. In particular, the addition of 1 molar magnesium chloride ($MgCl_2$) to attenuated poliovirus strains enabled vaccines to be stored at 4°C for three months or at 25°C for 25 days without significant loss of titre. Melnick et al. (*104*) found that vaccines stabilized with $MgCl_2$ which were subjected to 30°C for 21 days elicited an antibody response equal to that of ordinary vaccine maintained in the frozen state and thawed just prior to administration.

Other studies showed that 35% to 50% sucrose was effective in stabilizing attenuated polioviruses. Perkins and Magrath (*121*) and Magrath (*95*) concluded that both 1 M $MgCl_2$ and 50% sucrose were effective stabilizing agents. To achieve maximum virus stability it was necessary to prevent the rise in pH that occurred as CO_2 was lost from the container.

At present, most OPVs available on the market are stabilized with magnesium chloride, although some manufacturers produce sucrose-stabilized poliomyelitis vaccines. Recent studies appear to indicate that magnesium chloride is more effective than sucrose in increasing thermostability of OPVs. On exposure to 37°C for 8 days or to 25°C for 29 days the rate of potency loss in monovalent vaccines of all three types was higher in products stabilized with sucrose or buffer than in those stabilized with magnesium chloride (Figure 13).

Figure 13: Loss of potency of monovalent OPV stabilized with magnesium chloride, buffer and sucrose and stored at 37°C and 25°C



Note: b values refer to loss of titre per day (regression coefficients)

*Source: Mann GF et al. (*100*)*

A vaccine stabilized with magnesium chloride was more stable than one produced by the same manufacturer which was stabilized with sucrose at temperatures below 37°C (Table 10) (*117*). The better stabilizing effect of magnesium chloride has also been observed elsewhere (*109*).

It was concluded that the consistent use of magnesium chloride would help to increase the stability of OPV and to minimize reliance on the cold chain (*71*).

Table 10: Average losses of total virus content and half lives of oral poliomyelitis vaccines stabilized with sucrose and magnesium chloride and stored at various temperatures

Storage temperature (°C)	Time unit	Sucrose		Magnesium chloride	
		Titre loss*	Half life	Titre loss*	Half life
4	Month	0.11	6 months	0.02	20 months
20-25	Day	0.03	12 days	0.01	23.1 days
37	Day	0.15	1.9 days	0.16	1.8 days
45	Day	-	-	0.61	0.6 days

* Per time unit shown, in $\log_{10}$ $CCID_{50}$.

*Source: Peetermans JH, Colinet G (*117*).*

In a WHO collaborative study on 46 samples, 12 stabilized with sucrose and the others with $MgCl_2$, 83% of those stabilized with sucrose and 91% stabilized with $MgCl_2$ lost less than 0.5 $\log_{10}$ $CCID_{50}$ on incubation for 48 hours at 37°C. The mean loss was 0.34 $\log_{10}$ $CCID_{50}$, irrespective of the stabilizer (*157*). Buffered sucrose can evidently also be an efficient stabilizer provided that the pH is carefully controlled.

Poliovirus can be also stabilized against heat inactivation by adding fatty acids and related compounds. Incubation of type 1 Sabin poliovirus with myristic acid at 45°C for 30 minutes caused a 19% reduction in infectivity, while incubation without this fatty acid resulted in a 99% loss of infectivity (*36*). Thermal stabilization was also observed when poliovirus was incubated with hexanoic, octanoic or palmitic acids. The presence of these stabilizers during heating may prevent conformational changes in the capsid which render the virus non-infective.

Heavy water (D_2O) also has a stabilizing effect on polioviruses. With its strong hydrogen bonds it can protect protein against denaturing and enhance the thermostability of poliovirus strains. The infectivity of three D_2O- and $MgCl_2$-treated OPV strains exposed to 37°C for seven days remained within the limit of requirements, i.e., they did not lose more than 0.5 $\log_{10}$ $CCID_{50}$ (*166*). Despite the dramatic increase in the heat stability of OPV when heavy water was substituted for water, this avenue of research has not been pursued further, mainly for the following reasons:

- the available OPVs are sufficiently heat-stable to achieve their purpose of eradicating poliomyelitis;
- there would be disadvantages in licensing and introducing a new OPV while poliomyelitis eradication is in progress;
- VVMs are now used to monitor heat exposure in individual vials of OPV.

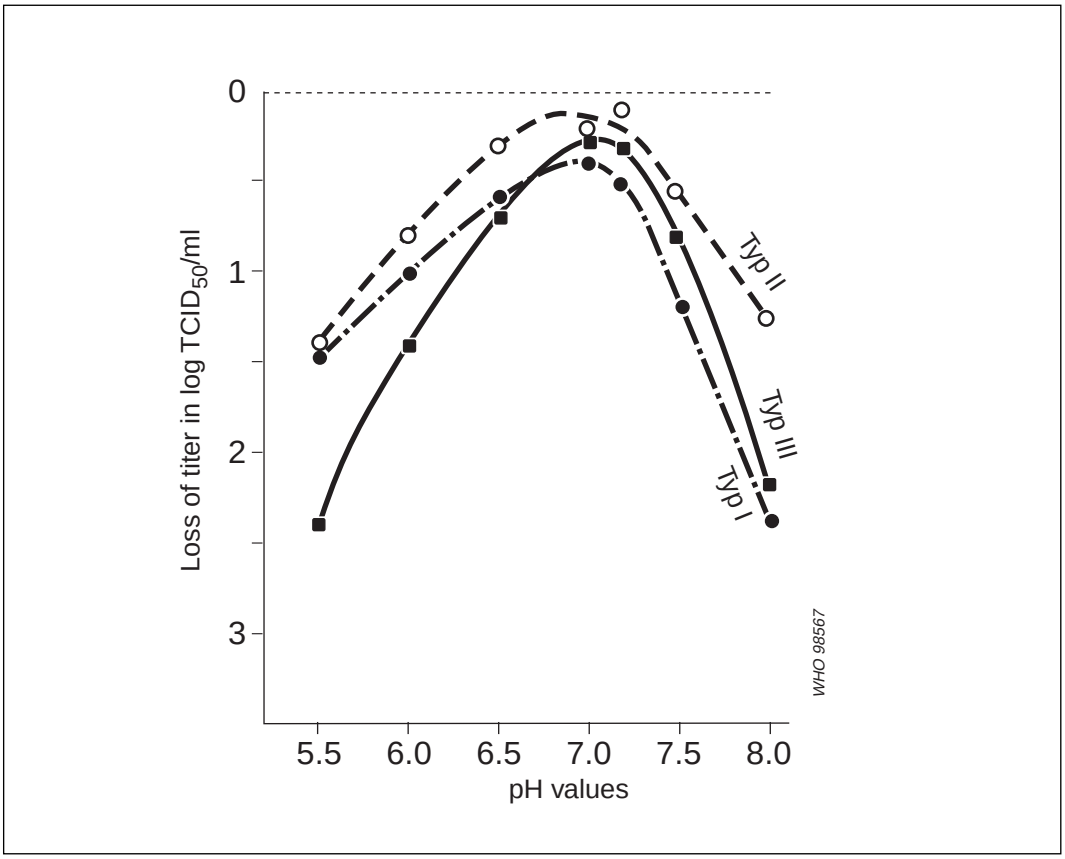
10.5.3 pH values of virus suspensions

Melnick and Wallis showed the importance of pH values in maintaining the stability of OPV (*105*). pH values increased in all tested vaccines, but to a much greater degree in loosely capped vials than in tightly stoppered ones. In tightly stoppered

vials, vaccines with an initial pH of 6.0-6.4 manifested no significant loss of infectivity after 20 days at 25-28°C, the titre falling only 0.1-0.2 $\log_{10}$ $CCID_{50}$. Samples in loosely capped vials lost their infectivity rapidly, evidently due to an increase in pH.

Similar observations were made by Mauler and Gruschkau, who studied monovalent poliomyelitis vaccines with pH values ranging from 5.5 to 8.0. After three days at 37°C, the highest losses were found at the extreme pH values of 5.5 and 8.0 (Figure 14) (*101*). Poliomyelitis vaccines were remarkably stable within the pH range 6.5-7.2.

Figure 14. Effect of pH on stability of attenuated polioviruses at 37°C for three days



*Source: Mauler R, Gruschkau H (*101*).*

A vaccine can be maintained in this pH range by preventing the loss of carbon dioxide from a bicarbonate-containing vaccine, keeping the air space above the vaccine to a minimum, and packing the vaccine in tightly stoppered containers.

10.6 Summary

OPV, as supplied by most manufacturers is stable for an extended period at -20°C, for over six months at 2 to 8°C, and for over 48 hours at 37°C. VVMs help to deal with the limitations of this vaccine in respect of thermostability.

Part III: Analysis of vaccine stability – other viral vaccines

11. Inactivated poliomyelitis vaccine

The capacity of poliovirus to produce neutralizing antibodies is destroyed by heat treatment, freeze-drying and the addition of merthiolate (thiomersal). As mentioned previously, the poliovirus component of a quadruple DTP-polio vaccine was not stable when merthiolate was used as a preservative. Beale and Ungar (*13*) demonstrated a rapid fall in potency of the poliovirus antigen in a quadruple vaccine preserved with merthiolate and sodium edetate and stored at 4°C. Another lot of quadruple vaccine without merthiolate but with half the amount of sodium edetate was stable for one year. These observations were recently confirmed with the high-potency inactivated poliomyelitis vaccine (eIPV), which was combined with DTP vaccine. Storage of eIPV at 4°C in the presence of merthiolate reduces the potency of type 1 poliovirus antigen to undetectable levels after four to six months. Type 2 and type 3 antigens are less markedly affected by exposure to merthiolate for eight months at 4°C (*126*). The incompatibility of eIPV and DTP vaccine preserved with merthiolate requires further study.

It appears that there are differences in heat stability between various inactivated poliovirus types, with type 1 being the most vulnerable. In the absence of preservative the type 1 component of trivalent inactivated poliomyelitis vaccine (IPV) deteriorates slowly after storage for two years at 4°C, while the two other types remain potent for 20 years. The D-antigen content for type 1 drops significantly after 20 days at 24°C and is undetectable after exposure to 32°C for the same period; no significant changes in D-antigen are observed for type 2 at either of these temperatures; type 3 remains stable for 20 days at 24°C but the D-antigen content drops significantly at 32°C (*110*).

All three types of IPV show satisfactory retention of potency when incorporated into combined vaccines and stored at 4°C for periods ranging from one year to over four years. These observations were made on DT-polio vaccine preserved with BC and adsorbed on aluminium hydroxide (*110*), and on aluminum phosphate DTP-poliomyelitis vaccine without preservative or with phenoxyethanol and formaldehyde as preservatives (*146*). Longer storage resulted in a decline in antigenicity, especially of the type 1 component (*110*).

At 37°C the D-antigen content of the poliomyelitis component of a quadruple vaccine decreases during storage but most of the potency remains after eight weeks. Type 3 seems to be the most stable component (*146*).

12. Mumps vaccine

The stability of both components of lyophilized measles-mumps vaccine are similar at 4°C, 23°C, 37°C and 45°C. At 37°C, the degradation rate is about 0.01 $\log_{10}$ per day for both components. Half lives are also similar: 4.7 and 5.4 days for the measles and mumps components respectively at 45°C; 12 and 13 days at 37°C, and 71 and 65 days at 23°C (*31*).

In the temperature range 20°C to 56°C, the mumps vaccine component in mumps-rubella and MMR vaccines have degradation rates comparable to those for monovalent mumps vaccine (*102*). The mumps component in Indian MMR vaccines show good stability at 37°C up to 21 days; during a 30-day exposure to 37°C, the mumps component of MMR vaccines lost 0.9 $\log_{10}$, i.e. about 0.03 $\log_{10}$ per day, and half lives were about 10 days (*73*).

13. Rubella vaccine

Freeze-dried monovalent rubella vaccine and the rubella component of measles-rubella, mumps-rubella and MMR vaccines show low degradation rates. At 37°C the average loss of titre ranges from 0.046 to 0.109 $\log_{10}$ $CCID_{50}$ per week (*102*). The rubella component of Indian MMR vaccine also shows good stability, the average titre loss per week being about 0.1 $\log_{10}$ $CCID_{50}$, and the half life being more than two weeks (*73*). The rubella component seems to be more stable than the other components of combined virus vaccines.

The WHO thermostability requirements for mumps and rubella vaccines are similar to those for measles vaccine. At least three containers of monovalent or MMR vaccine are tested by incubation at 37°C for seven days, at the end of which each monovalent vaccine or individual vaccine component is titrated in PFUs or $CCID_{50}$ after selective neutralization, as necessary, of the other components. The geometric mean infectious virus titre must equal or exceed the required minimum number of infective units per human dose (3 $\log_{10}$), and the geometric mean virus titre must not have decreased by more than 1 $\log_{10}$ infective units during incubation (*153*).

14. Hepatitis A vaccine

The isolation and adaptation of hepatitis A virus to cell culture opened the way to the development of vaccines. Harvests of hepatitis A virus, multiplied in cell culture, are clarified, purified and concentrated and then inactivated by formaldehyde. Aluminium hydroxide or aluminium phosphate is used as an adjuvant.

ADTs performed on vaccine lots at release and after storage for 15 months in a refrigerator indicate no loss of immunogenicity at 37°C for up to three weeks (*116*). Hepatitis A vaccine kept for one week at 37°C produce an immune response in seronegative persons which does not differ significantly from that induced by properly stored vaccine (*163*).

15. Japanese encephalitis vaccine

15.1 Freeze-dried vaccine

At present, two types of formalin-inactivated Japanese encephalitis (JE) vaccines are in use. One, derived from mouse brain, is used in India, Japan, Republic of Korea, Thailand, and other countries. The other is derived from primary hamster kidney cell culture and is used in China.

A freeze-dried JE vaccine produced in India is stable, undergoing falls in potency of only 4.7% in 52 weeks at 4°C and of 8.7% in 28 weeks at 22°C. Degradation is more rapid at higher temperatures: potency declines by 14% and 24% during 18 weeks at 37°C and 40°C respectively (*58*).

15.2 Reconstituted vaccine

After reconstitution the vaccine is still stable at 22°C. There is a 1% drop after two weeks, and a fairly rapid deterioration in potency in four weeks at 37°C and 40°C (*58*).

16. Rabies vaccine

The solution to the problem of rabies vaccine safety is in the development of vaccines prepared from rabies virus grown in tissue culture free from neuronal tissue (*123*). Since 1976, use of human diploid cell vaccine (HDCV) has become general for pre- and post-exposure immunization of humans. HDCV vaccine evokes much better immune responses than did vaccines such as DEV (prepared from virus propagated in embryonated duck eggs), or vaccines prepared from suckling mouse brain (Fuenzalida vaccine) or adult sheep or rabbit brains (Semple vaccine).

HDCV in its lyophilized form is a very stable vaccine; it retains its potency for at least 24 months at temperatures between 2°C and 8°C and for one month at 37°C (*112*). Another HDCV vaccine was stable for at least 18 months at -20°C and + 4°C, and withstood exposure to 37°C and 60°C for three months (*97*). A lyophilized human diploid cell strain rabies vaccine that was dispatched, transported and stored at 26–36°C for up to 11 weeks, has stimulated similar antibody responses in Pakistani medical health staff to those produced by HDCV vaccine transported and stored at 2–13°C (*111*).

Human diploid cell rabies vaccine is difficult and expensive to produce. There have been intensive efforts worldwide to produce vaccines at a low cost that can meet or improve on the levels of safety and efficacy achieved with HDCV (*123*). The new vaccines include: the purified chicken embryo cell vaccine (PCEV) developed in the Chiron Institute, Germany (formerly Behringwerke), which was stable for three months at 37°C (*14*): primary Syrian hamster kidney cell culture vaccine (PHK), developed in the Poliomyelitis Institute in Moscow, Russian Federation and used in China; the Vero cell line vaccine (PVRV) developed in the Pasteur-Merieux-Connaught Institute, France; and purified duck embryo vaccine (PDEV) developed in the Swiss Serum and Vaccine Institute in Switzerland. All are likely to have much better stability than the former vaccines prepared on neural tissues.

Part IV: Analysis of vaccine stability – other bacterial vaccines

17. Meningococcal polysaccharide vaccine

Purified polysaccharides, and especially group A polysaccharides, are unstable at ambient temperatures because of depolymerization. Polysaccharide antigens readily depolymerize and their relative molecular mass diminishes when they are exposed to ambient temperatures. The degree of polymerization is therefore a useful indicator for assessing both immunogenicity and thermal stability.

Storage at -20°C was recommended for the early polysaccharide vaccines in group A. At that temperature, the rate of depolymerization is negligible. The immunogenicity of meningococcal vaccine is related to the molecular size of the protective antigens, polysaccharides A and C; the antibody response increases with the molecular weight. The discovery, that the replacement of sodium chloride by lactose as a menstruum for lyophilization stabilizes polysaccharide vaccines against thermal depolymerization, represented a major step in achieving more stable vaccines (*143, 151*). These vaccines are supplied in freeze-dried form. The addition of a stabilizer and achievement of a low moisture content have greatly improved their thermal stability.

Stabilized meningococcal vaccines in the lyophilized state can be stored at refrigerator temperatures for two years (*8, 9*). Group A polysaccharide vaccine was unaffected by being kept at 20–25°C for 12 days or at 35°C for 3 days (*9*). Group A+C vaccine from one manufacturer, stored at 22°C for 18 months, showed very little depolymerization; at 45°C the group A component reached a critical level of depolymerization after 4 weeks, while the group C component was stable for 8-10 weeks (*8*).

A vaccine reconstituted with diluent containing 0.25% phenol was reported to be stable when stored at -20°C for two months, at 4°C for four weeks, at 25°C for two weeks, or at 37°C for four days (*8*). Despite its relative stability, reconstituted vaccine should be kept at refrigerator temperatures and should be discarded if not used during the day on which it is reconstituted (*151*).

18. *Haemophilus influenzae* type b vaccine

The stability of conjugated polysaccharide vaccines, including *Haemophilus influenzae* type b (Hib) vaccine, may depend on the impact of adverse factors on the strength of the linkage between the polysaccharide and the protein carrier. Although

there are few data on these vaccines, preliminary results suggest that the lyophilized Hib vaccine (tetanus toxoid conjugate vaccine containing purified polyribosyl-ribitol-phosphate capsular polysaccharide, PRP-T) is stable at refrigerator temperatures for 36 months and at 25°C for at least 24 months. Reconstituted monovalent Hib vaccine or reconstituted Hib vaccine combined with other vaccines (DTP, DTP-HB, or DTP-IPV) should be destroyed after an immunization session or within six hours. Liquid monovalent Hib or liquid Hib-DTP vaccines are stable at refrigerator temperatures for 24 months. In multidose formulation, liquid Hib and Hib-DTP vaccines may be used at a subsequent session, even if they have been opened, according to the WHO Policy Statement on the use of opened vials of vaccine in subsequent immunization sessions (*161*).

19. Typhoid vaccines

In the past, parenteral killed whole cell typhoid vaccines have been widely used but they frequently caused local pain and swelling, fever, headache, and malaise.

In the most recent years, three typhoid vaccines were used: parenteral Vi polysaccharide-carrier protein conjugated vaccine, attenuated *Salmonella typhi* strains used as live oral vaccines, and inactivated whole cell oral vaccines (*88*).

The Vi polysaccharide vaccine is highly stable and does not require a cold chain even in tropical conditions. This is a distinct advantage of this vaccine.

Live oral vaccine contains the Ty21, a mutant of *S. typhi*, and should be stored at + 4°C. The shelf life of lyophilized vaccine is dependent on residual moisture content and maintenance of the cold chain. Nearly half of compliance errors in American travellers were due to improper storage (*32*). Vaccine failures in Swiss travellers have been associated with vaccine which was not kept in a refrigerated state (*67*). Prolonged storage at room temperature resulted in progressively lower viable counts over time, although all tested lots evaluated after storage for seven days at 20°C to 25°C met potency requirements. Three lots of the vaccine stored at 37°C for 12 hours also maintained potency (*32*).

20. Cholera vaccines

Although parenteral killed cholera vaccines are not recommended by WHO to any persons of any age, they are still available commercially in many countries.

Recently, killed oral whole cell vaccine combined with B subunit (WC/BS vaccine) has been developed in an attempt to stimulate local intestinal mucosal immune responses in a manner similar to that induced with natural exposure (*127*). The storage requirements for oral killed vaccine are similar to these needed for the former parenteral vaccines (the vaccine is stable for three years when kept in the refrigerator at 2-8°C).

Another recently developed vaccine is a live oral CVD103 HgR vaccine. Because the vaccine strain is live, the viability of bacteria must be preserved while in storage and an effective cold chain is likely to be needed.

Part V: Final conclusions

The stability of vaccines varies considerably. They can be ranked by their resistance to storage at elevated temperatures, with diphtheria and tetanus toxoids and hepatitis B vaccine showing the highest thermostability, freeze-dried measles, yellow fever and BCG vaccines occupying the middle position and oral poliomyelitis vaccine being the most fragile. Reconstituted vaccines against measles, yellow fever and tuberculosis (BCG) are unstable vaccines; they should be used as soon as possible after reconstitution, be kept in a ice bath during the immunization session and should be discarded at the end of the session.

The data presented show that some vaccines can withstand a long period of exposure without a significant loss of potency. The high resistance of tetanus toxoid and hepatitis B vaccine to heat may warrant studies on the use of these vaccines without refrigeration. They could retain sufficient potency during short-term exposure to heat when used in outreach programmes for the immunization of women of childbearing age against tetanus or immunization of children against hepatitis in areas where the cold chain cannot be maintained.

At present, there are three reports on the use of tetanus toxoid and hepatitis B vaccine outside the cold chain, without refrigeration. A study was performed in Indonesia with a prefilled, single-use injection device, "UniJet". The device was stored at ambient temperature for up to one month in midwives' homes. The study showed that the midwives used the device properly and safely to administer hepatitis B vaccine to the infants and tetanus toxoid to their mothers. Injection recipients and midwives expressed a strong preference for a single-use device over a standard syringe (*140*).

In a study in Bolivia, the acceptability of UniJet, prefilled with tetanus toxoid and kept by midwives at their homes without refrigeration, was also very high. The device requires no assembly, eliminates the steps to fill the syringe and adjust the vaccine dose and does not require ice and cold storage containers. With its ability to improve safety, simplify logistics, and reduce wastage rates, the device represents a potential cost-effective strategy for outreach immunization (*108*). In China, the seroconversion rate in infants immunized by village midwives with hepatitis B vaccine stored without refrigeration did not differ significantly from the rate observed in children immunized by village doctors with the vaccine stored in refrigeration (*7*).

However, each exposure to elevated temperature results in some degradation of the vaccine, even if the remaining potency is still above the level considered to be the minimal immunizing potency. Furthermore, each exposure to ambient temperature

has a cumulative impact on vaccine potency. Vaccines in peripheral health units could not withstand the temperatures mentioned above if their potency had already been compromised by previous breaks in the cold chain. The data presented may be useful for those involved in immunization activities at central and provincial levels who have to make decisions about vaccines exposed to elevated temperatures.

All vaccines should be routinely stored at the temperatures recommended by manufacturers and national immunization programmes. The cold chain remains a highly vulnerable point for these programmes in developing countries with tropical climates. Developed countries with temperate climates can have similar problems. In all countries, systems of refrigeration, temperature-monitoring and record-keeping are required to make sure that each vial of vaccine is maintained under appropriate conditions and that it is used before the expiry date shown on the label.

A summary of information on the stability of vaccines stored at various temperatures is presented in the following summary tables.

Part VI: References

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Annex: Summary tables of vaccine stablity

Table A: Stability of vaccines commonly used in national immunization programmes

Vaccine[1]	Storage temperature (°C)			
	0-8	22-25	35-37	Over 37
Tetanus and diphtheria toxoids as monovalent vaccines or components of combined vaccines[2]	Stable for 3-7 years	Stable for months	Stable for weeks	At 45°C: stable for 2 weeks; At 53°C: loss of potency after few days; At 60-65°C: loss of potency after few hours
Hepatitis B vaccine[2]	Stable for 2 - 4 years	Stable for months	Stable for weeks	At 45°C: stable for days
Measles vaccine[3]	Stable for 2 years	Retains satisfactory potency, up to 50%, for at least 1 month.	Retains satisfactory potency for at least one week, but may lose 20% and 50% of potency for 1-4 day and 2-6 day exposure, resp.	At 41°C: 50% loss of potency after 2-3 day exposure; At 54°C: 80% loss of potency after one-day exposure.
Yellow fever vaccine[3]	Stabilized vaccines stable for 2-3 years	50% loss after 3-10 month exposure	50% loss after 10-20 day exposure	
Pertussis vaccine[2]	Stable for 18-24 months, in spite of continuous slow decrease in potency	Stability varies: some vaccines stable for 2 weeks	Stability varies. Some vaccines lose 50% of potency during storage for one week	At 45°C: about 10% loss of potency per day; At 50°C: rapid loss in potency
BCG vaccine[3]	Stable for one year	Stability varies: 20% to 30% loss of viability during 3-month exposure	Stability varies. 20% loss of viability during 3-14 day exposure	Unstable. At 70°C: 50% loss during 30 minute exposure
Oral poliomyelitis vaccine[3]	Stable for 6-12 months	Some vaccines may retain titres for 1-2 week exposure	Unstable. VVMs in use. Loss of satisfactory titre in 1-3 days	Very unstable. At 41°C: 50% loss in one day. At 50°C: loss of satisfactory titre after 1-3 hour exposure

1. Data refer to freeze-dried measles, yellow fever and BCG vaccines; other vaccines are presented in a fluid form. Reconstituted vaccines lose their potency quickly and they must be discarded at the end of an immunization session. Reconstituted BCG vaccine contains no bacteriostatic agent and there is a risk of contamination. Reconstituted yellow fever vaccine should be administered quickly (up to one hour) after reconstitution. If the vaccine can be kept continuously in an ice bath, the reconstituted vaccine can be used within one immunization session. It must be discarded after the session.

2. Vaccines adsorbed on aluminium salts. They should never be frozen.

3. Optimal long-term storage is at -25°C or less. Diluent should be kept separately and should not be frozen.

Table B: Stability of other bacterial and viral vaccines

Vaccine[1]	Storage temperature (°C)			
	0-8	22-25	35-37	Over 37
Inactivated poliomyelitis vaccine	Stable for 1 to 4 years	Decline of D-antigen[1] content of type 1 in 20 days	Loss of D-antigen of type 1 content in some vaccines	No precise data available
Meningococcal polysaccharide vaccine	Stable for 2 years	Group A vaccine: stable for 12 days; group A+C stable for months	Half life[2]: 4 weeks	No data available
Human diploid cell rabies vaccine	Stable for 3.5 years	Retained immunogenicity when dispatched, transported and stored for up to 11 weeks	Stable for 4 weeks	No data available
Japanese encephalitis vaccine	Stable for one year; about 5% loss in potency during 52-week storage	Stable for 20 weeks, about 9% loss in potency during 20-week storage	Stable for 6 weeks; about 14% loss in potency during 18-week storage	At 40°C: about 10% loss in potency after 2-week storage and 27% loss after 6-week storage
Live oral typhoid Ty21a vaccine	Needs refrigeration. Shelf life depends on residual moisture content	Prolonged storage resulted in progressively lower viable counts	Rapid decrease in viable count. Retains minimum potency for 12-hour exposure	No data available

1. D-antigen content is measured *in vitro* by the ELISA test. IPV is standardized in D-antigen units; enhanced-potency IPV contains 40, 8, and 32 D-antigen units of types 1, 2 and 3 respectively.

2. Half life: the time at which 50% loss of original potency occurs.