STABILITY OF VACCINES

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1. INTRODUCTION

Immunization services in developing countries are preventing each year over 350,000 children from becoming paralyzed from poliomyelitis and over two million deaths from measles, neonatal tetanus and pertussis (117). The progress in the immunization programmes is due in part to training of staff in the proper storage and transport of vaccines used in the Expanded Programme on Immunization (EPI) and to improvements in the cold chain system.

However, in many areas vaccines are still not being stored and transported properly. Questions are often raised concerning what to do with a stock of vaccines exposed for varying periods of time to elevated temperatures. There is no simple and cheap method which can be used in the field to assess whether a vaccine exposed to ambient temperature has retained at least minimum required potency. This may be determined only by laboratory assays. However, such assays are costly, results are often delayed for several months and only a large number of doses (from 2,000 for poliomyelitis and measles vaccines to 200,000 for DPT vaccine) justifies sending the vaccine for retesting (35).

Knowledge concerning the stability of a vaccine, and especially the rate at which it loses its potency at a given temperature, can be helpful in deciding whether the vaccine should be destroyed, sent for retesting or used. Since the previous review (31) much data have become available concerning the stability of vaccines stored and transported at ambient temperatures. These data however, are difficult to summarize because techniques used to assess the stability of vaccines are not standardized.

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. A shorter and more feasible procedure is the accelerated degradation test (ADT). The principle of the ADT is to subject samples to a range of elevated temperatures at which denaturation will occur more rapidly than at normal storage temperature. Thus, a significant and readily detectable degradation may be induced in a relatively short time. By measuring the degradation rate at higher temperature and by assuming that this rate follows a fixed law of dependency on temperature (the Arrhenius equation), extrapolation may be then made to the lower temperatures at which a vaccine is to be stored (106).

ADT studies may predict degradation rates with precision which differs considerably, as they are affected by the range of temperatures used, the number of a samples tested and the design of the ADT.

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 titer 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 a large number of animals with the potency expressed in arbitrary 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 deterioration of the vaccine unless marked deterioration has occurred (89).
Vaccines and toxoids are made up of proteins, nucleic acids and carbohydrates, which undergo changes on exposure to heat. The degradation rate of any vaccine is determined by the storage temperature; the higher the temperature the more rapid and larger the degradation. There are considerable differences between degradation rates for EPI vaccines. However, the degradation rate \( b \) is not the only factor determining the residual potency \( Y_t \) of a vaccine. Two others factors are the time \( T \) at which a vaccine is stored at a given temperature and the initial potency of a vaccine \( Y_0 \). The relationship between these three factors may be expressed by the following formula:

\[
Y_t = Y_0 - bT
\]

The usefulness of this formula is limited by the fact that a programme manager or a storekeeper does not know the initial potency of a vaccine. However, the knowledge of the degradation rate characteristics for various temperatures and of the time at which a vaccine was exposed to a given temperature may be helpful for a worker in deciding what to do with suspect vaccine.

This paper reviews current information concerning the stability of vaccines. The main emphasis has been put on vaccines currently included in the global EPI but some information is also provided on viral vaccines against rubella, mumps, hepatitis B, yellow fever and rabies and bacterial vaccines against meningococci, cholera and typhoid.
2. DIPHTHERIA AND TETANUS TOXOIDS

Adsorbed diphtheria and tetanus toxoids in monovalent form or as components of combined vaccines are the most stable of the EPI vaccines. They are stable at elevated temperature even for long periods of storage but may change their appearance and lose potency when frozen.

The potency of tetanus components of adsorbed DPT or DPT-polio vaccines has not shown significant change at temperatures of $+4^\circ$C to $+8^\circ$C for 3 to 7 years (56, 58, 92, 97). The shelf life at the temperatures recommended usually by manufacturers ($+2^\circ$C to $+8^\circ$C) depends on the nature of the vaccine: the validity period is usually longer for monovalent toxoids or combined diphtheria tetanus vaccine (usually 3 years) than for DPT or DPT-polio vaccines (18-24 months). In combined vaccines, limiting factors are pertussis or poliomyelitis components.

The toxoid components of DPT or DPT-polio vaccines show an insignificant decrease of potency when stored for 1.5 years at $18^\circ$C (97), for 6 to 12 months at $24^\circ$C (101), and for 2 to 6 months at $37^\circ$C (58, 92, 101).

A storage at $37^\circ$C for up to 22 weeks, however, resulted in 50% reduction of the original potency of diphtheria and tetanus components of DPT-polio vaccines of one producer (92). In some other DPT vaccines, the tetanus toxoid component showed more accentuated deterioration when stored for 45 days at $22^\circ$C and $35^\circ$C; the daily losses were about 0.5% and 1% respectively (Figure 1).

Figure 1
Potency of the tetanus component of DPT vaccine stored for 45 days at various temperatures (1U per single human dose, average from two lots tested)

According to (59)
Diphtheria and tetanus toxoid components of some DPT vaccines can withstand a several week exposure to higher temperatures. No significant loss of the potency of either of the toxoid components was observed when DPT vaccine from one producer was stored at 45°C for 2 to 4 weeks. However, a storage at that temperature for 8 weeks resulted in some 40% loss in potency (58). In some other DPT vaccines the deterioration process was more rapid; at 45°C the loss of potency of the tetanus component was 5% per day in the first 2 week period and 1% per day during the next month of storage (59).

At temperatures higher than 45°C, the degradation of toxoid potency is accelerated. Monovalent adsorbed tetanus toxoid, tested in the accelerated degradation test lost 20% and 50% of its initial potency after four and eight day exposures, respectively, to a temperature of 53°C (Table 1). A high temperature of 60°C results in quick (3 to 5 hours) destruction of tetanus or diphtheria toxoids (22, 100).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time of exposure (hours)</th>
<th>Remaining potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>96</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>53</td>
</tr>
<tr>
<td>55</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>288</td>
<td>35</td>
</tr>
<tr>
<td>65</td>
<td>3</td>
<td>20</td>
</tr>
</tbody>
</table>

The observations discussed above refer to the potency of toxoids as determined in animal tests. A long exposure to high temperature may result in some changes in the physical characteristics of the aluminium compound which are not revealed by animal potency tests. The aluminium hydroxide adjuvant showed symptoms of "ageing" in the form of morphological and structural changes when stored as a single compound or as the adjuvant of D, DT and DPT vaccines (3, 118, 119). A continuous decline in its ability to adsorb Congo red dye was observed during a storage at temperature of +4°C to +10°C for 5.5 years. Additionally, electron microscope and rentgenographic studies showed that morphological and structural changes progress more rapidly at 10°C than at 4°C (118, 119).
The freezing point for adsorbed DPT vaccines is between \(-5^\circ C\) and \(-10^\circ C\) (32). Adsorbed vaccines, monovalent or combined, change their physical appearance after freezing. The occurrence of agglomerates, flocules or other granular matter results in an increase of the sedimentation rates (7, 32, 76, 97). The size of the granules seems to increase on repeated freezing and thawing and the granules to not form a uniform suspension even on vigorous shaking (76). Freezing of the aluminium hydroxide gels caused extensive morphological changes visible in electron microscope studies, and this contributes to increasing the sedimentation rate (3).

These physical changes induced by freezing are the basis for the "shake test" which can be useful in detecting adsorbed vaccines which have been frozen (32). The performance of the "shake test" is easy: the vaccine container is vigorously shaken and the content is examined for physical changes. Then, the extent of sedimentation is checked after 30 minutes. The presence of granular forms or flocules in the vaccine when it is shaken or a sediment formed at the bottom of the container within 30 minutes with clear liquid above, suggest that the vaccine has been frozen (32).

Freezing can cause reduction of tetanus toxoid potency. Results have been reported which suggest slightly different behavior of frozen products, depending on the composition of vaccine containing tetanus toxoid. Out of 5 DPT vaccines stored for 12 hours at \(-30^\circ C\), the tetanus component of two vaccines showed some 30\% decrease in potency. There was no potency decrease in vaccine stored at \(-5^\circ C\) to \(-10^\circ C\). However, the potency of the tetanus toxoid component of adsorbed DT vaccine was reduced after freezing at both temperatures: \(-5^\circ C\) and \(-30^\circ C\) (32).

Frozen monovalent tetanus toxoid, used in young military recruits, especially toxoid frozen four times, stimulated a lower mean response and a lower proportion of high titers than the unfrozen product. All persons immunized with frozen toxoids, however, acquired protective levels of tetanus antitoxin. Freezing does not seem to affect the immunogenicity of unabsorbed toxoid (which remains less immunogenic than the adsorbed product) (Table 2.)

Table 2. Immune response of military recruits immunized with frozen and unfrozen adsorbed tetanus toxoid
According to Menon et al. (76)

<table>
<thead>
<tr>
<th>Toxoid</th>
<th>Treatment</th>
<th>10 days after 1st dose</th>
<th>10 days after 2nd dose</th>
<th>10 days after 3rd dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% &gt; 0.01 IU/ml</td>
<td>mean in IU/ml</td>
<td>% &gt; 1.0 IU/ml</td>
</tr>
<tr>
<td>Adsorbed on AlPO4</td>
<td>unfrozen</td>
<td>50</td>
<td>0.07</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>frozen 1x</td>
<td>47</td>
<td>0.07</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>frozen 4x</td>
<td>46</td>
<td>0.05</td>
<td>77</td>
</tr>
<tr>
<td>Non-adsorbed</td>
<td>unfrozen</td>
<td>50</td>
<td>0.04</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>frozen 1x</td>
<td>50</td>
<td>0.05</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>frozen 4x</td>
<td>54</td>
<td>0.06</td>
<td>30</td>
</tr>
</tbody>
</table>
Adsorbed DPT, DT or TT vaccines which are suspected of having been frozen should be examined for physical changes. Vaccines exhibiting such changes should be discarded. The amount of antigen administered in a non-homogenous vaccine can be subject to great variation and the administration of such a vaccine may be associated with a reduced immune response and/or with an increased incidence of local reactions.
3. PERTUSSIS VACCINE

Stability studies on pertussis vaccine are hampered by the lack of a simple, cheap and repeatable test for pertussis vaccine potency. The potency test recommended by WHO (109) is technically difficult and requires highly qualified staff and a large number of a selected strain of mice. The results are subject to wide biological variation and it is difficult to obtain precise data on the deterioration of the potency of vaccine exposed to elevated temperatures unless marked changes in vaccine potency can be observed.

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

1. the temperature (7, 25, 34, 57, 58, 59, 92, 93);
2. the form of the vaccine: monovalent vaccine vs pertussis component of DPT vaccine (7, 25, 48, 54);
3. the method of its inactivation (45, 51, 54);
4. the strain of B pertussis organism (20) and
5. the nature of adjuvant or preservative (83, 92).

3.1 Influence of temperature on vaccine potency and toxicity

The potency of the pertussis component of DPT vaccine depends on the temperature of storage; diminution of potency may be caused by both high temperatures and freezing.

Stored in a refrigerator between +4°C and +6°C the pertussis component of DPT or DPT-polio vaccines appears to have satisfactory potency over a period of two years (56, 58, 92). However, even with optimal storage conditions a continuous decrease in potency of the pertussis component is observed during long periods of storage. DPT vaccines with an average initial estimated potency of 8.5 IU per single human dose declined to less than 4 IU per dose after 46 months (56). Similarly the potency of the pertussis component of DPT polio vaccines declined from 5.2 - 8.6 IU/ml to 1.2 - 1.6 IU/ml after 3 years storage at 40°C (58).
Table 3. Stability of the pertussis component of DPT vaccines at various temperatures

<table>
<thead>
<tr>
<th>Storage temperature (°C)</th>
<th>Authors (year)</th>
<th>Estimated potency loss per day in %</th>
<th>Time of storage and time used for calculation of degradation rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 - 8</td>
<td>Caizer et al. (1978)</td>
<td>0.06</td>
<td>6 years</td>
</tr>
<tr>
<td></td>
<td>Kumar et al. (1982)</td>
<td>0</td>
<td>45 days</td>
</tr>
<tr>
<td></td>
<td>Andrescu et al. (1985)</td>
<td>0</td>
<td>12 - 18 months</td>
</tr>
<tr>
<td></td>
<td>Gupta et al. (1985)</td>
<td>0.01</td>
<td>90 days, 15 - 90 days</td>
</tr>
<tr>
<td></td>
<td>Kreeftenberg (1989)</td>
<td>0.05 - 0.06</td>
<td>3 years</td>
</tr>
<tr>
<td>22 - 25</td>
<td>Kumar et al. (1982)</td>
<td>0.31</td>
<td>45 days, 0 - 45 days</td>
</tr>
<tr>
<td></td>
<td>EPI, Yugoslavia (1985)</td>
<td>0.41</td>
<td>140 days, 40 - 140 days</td>
</tr>
<tr>
<td></td>
<td>Andrescu et al. (1985)</td>
<td>0</td>
<td>30 d. followed by 10 m at 40°C</td>
</tr>
<tr>
<td></td>
<td>Gupta et al. (1987)</td>
<td>0.26</td>
<td>90 days, 15 - 90 days</td>
</tr>
<tr>
<td>30</td>
<td>EPI, Yugoslavia (1985)</td>
<td>1.80</td>
<td>90 d., rapid decrease 0-15 d. slow decrease 30 - 90 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>35 - 37</td>
<td>Ramahorst &amp; Wezel (1976)</td>
<td>3 - 6*</td>
<td>56 days, 0 - 7 days</td>
</tr>
<tr>
<td></td>
<td>Rao et al. (1985)</td>
<td>1.2</td>
<td>90 days, 0 - 15 days</td>
</tr>
<tr>
<td></td>
<td>EPI, Yugoslavia (1985)</td>
<td>5.2</td>
<td>60 days, 0 - 20 days</td>
</tr>
<tr>
<td></td>
<td>Gupta et al. (1987)</td>
<td>2.4</td>
<td>90 days, 0 - 15 days</td>
</tr>
<tr>
<td></td>
<td>Kreeftenberg (1989)</td>
<td>5.5</td>
<td>56 days, 0 - 7</td>
</tr>
<tr>
<td>46</td>
<td>Kreeftenberg (1979, 1989)</td>
<td>6.7</td>
<td>56 days, 0 - 7 days</td>
</tr>
<tr>
<td></td>
<td>EPI, Yugoslavia (1985)</td>
<td>10.6</td>
<td>20 days, 0 - 4 days</td>
</tr>
</tbody>
</table>

* Two DPT-Polio vaccines with different preservatives

In another study, the average annual lose of potency of the pertussis component of DPT vaccines was estimated at 0.35 IU per human dose; the potency of vaccines reached a minimum 4 IU per dose after a storage of 6 years at a temperature of +40°C (25, 26, 51).

Since there is a certain loss of potency during storage, only vaccine with sufficient excess in initial potency should be released to ensure a minimum potency of 4 IU per single dose during the whole period of validity.

The impact of ambient temperatures on the potency of the pertussis component of DPT vaccine is shown in Figure 2 and Table 3. At 22°C - 25°C the potency of pertussis vaccine remains above 80% of its original value for 2 to 8 weeks. It decreases gradually thereafter with an estimated degradation rate of 0.3% - 0.4% per day.
Figure 2.
Potency (in percentage of the initial value) of the pertussis component of DPT vaccine kept for various lengths of time at different temperatures According to (34).

At 37°C, the process of vaccine degradation is more dramatic and seems to be biphasic: at the beginning the potency declines more rapidly, with an estimated degradation rate between 1% and 6%, while later on the rate of degradation slows (34, 45, 58, 92).

With storage at 45°C - 46°C the decline during the first few days of exposure is high, reaching some 10% per day. Fifty percent loss may occur after only a 4 to 7 day exposure (34, 57), and storage at 50°C to 56°C brings about rapid and complete loss of potency of the pertussis component (14, 48).

Some authors have claimed higher resistance of the pertussis component of DPT vaccines to elevated temperatures than that discussed above (14, 59, 93, 101). The reason(s) for such better thermo-resistance of these vaccines is unknown.
Freezing may impair the potency of pertussis vaccines. The potency of the pertussis component of DPT vaccines submitted to freezing at -20°C for 15 days loses more than 50% of its initial value. The potency of the pertussis component was more impaired by freezing than by storage at elevated temperatures (Fig.3). Results of a WHO Collaborative Study, in which adsorbed DPT vaccines from 5 different manufacturers were kept for 12 hours at -5°C to -10°C and at -20°C to -30°C, showed that three vaccines experienced significant losses in their pertussis component potency at both ranges of temperature (32).

There is no evidence that the toxicity of pertussis vaccine increases with storage, as measured by the mouse weight-gain and histamine sensitizing tests (7, 20, 46). In fact, vaccine samples kept at 25°C and 35°C for 4 weeks to 3 months showed reduced toxicity (20, 46).

3.2 Monovalent pertussis vaccines versus pertussis component of combined vaccines

Results of studies on the stability of monovalent pertussis vaccines differ considerably. In an early study, Kendrick et al. (54) found that monovalent non-adsorbed pertussis vaccines retained mouse-protective properties for as long as eight to ten years when stored at 5°C to 10°C. However, the monovalent pertussis vaccines studied by Ikiz et al. (49) seemed to be rather unstable at 4°C; after storage for 18 months some samples lost from 58% to 87% of their initial potency.

B. pertussis bulk suspensions seem to deteriorate more rapidly at 4°C during the first year of storage than the pertussis component of DPT vaccines adsorbed on aluminium phosphate (Figure 4).

The better stability of pertussis vaccine as a component of DPT vaccine probably results from the protective effect exerted by the proteins of toxoids and the aluminium ions present in the triple vaccine. The influence of aluminium ions will be discussed in chapter 3.5.
**Figure 3.**

Immunogenicity of monovalent pertussis vaccine and the pertussis component of DPT vaccine stored at various conditions

According to Andresco et al. (7)

Immunogenicity expressed as relative potency in comparison with the national reference preparation. The relative potency of 0.5 is equal to 4IU per single dose.

![Graph showing relative potency of pertussis vaccine and pertussis component at different storage conditions.](image)

**3.3 Methods of inactivating B. pertussis organisms**

Early studies on the stability of pertussis vaccines prepared from cultures grown and killed by various methods suggest that no inactivating agent (merthiolate, phenol, formalin or heat) stands out as definitely superior in this respect. With prolonged storage, however, the phenol and formalin-killed vaccines become dark in color and difficult to resuspend while vaccines killed by merthiolate and by heat show little change in appearance.

Early observations by Kendrick et al. (54) have been confirmed by Gupta et al. (45) who studied the stability of the DPT pertussis components prepared with different methods of inactivation (heat, formaldehyde, glutaraldehyde, thiomersal, or acetone treatment). Stability tests performed after the storage of vaccines at 40°C to 80°C, 25°C and 35°C (Figure 5) for 90 days showed no difference in the stability of pertussis vaccines attributed to the inactivating agents used in the manufacture of pertussis preparations.
Gupta's study shows 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 differed considerably; thiomersal inactivated vaccines (TIP) having the highest potency and acetone-treated vaccines (not shown in Figure 5) being of substandard potency. It may be noted that given the variability of the test, the differences seen in degradation rates in figure 5 are not significant.

**Figure 4.**
Potency (in % of the initial value) of pertussis vaccine bulk and the pertussis component of DPT vaccine stored for one to eight years at 40°C.

3.4 **Strains of B.pertussis organisms**

Data on the influence of different pertussis strains on the stability of pertussis vaccine are scarce. Vaccines prepared from six different B.pertussis strains showed some differences in stability but the numbers were too small to make a clear distinction between stable and unstable strains. A strain which resulted in a vaccine with higher stability, however, retained both LPF and HSF activities longer than vaccines prepared from other strains (20).

3.5 **Influence of preservative and adjuvant**

It was known from the early studies of Edsall et al. (29) and Pittman (91) that a significant loss of potency of the pertussis vaccine component of quadruple DPT polio vaccine may occur when benzethonium chloride (BC) is used as preservative.
Figure 5.

Potency of the pertussis component of DPT vaccines stored at 35°C. The pertussis component was inactivated by heat (HIP), formaldehyde (FIP), glutaraldehyde (GIP) and thiomersal (TIP). According to Gupta et al. (45)

BC was introduced as a preservative to replace merthiolate when it was shown that the latter inactivated the poliovirus component in the quadruple vaccine. BC is a quaternary ammonium compound which acts probably by attachment to the negatively charged sites on the cell surface of B. pertussis organisms.

Olson et al. (83) confirmed that pertussis vaccine preserved with BC was inactivated during storage. The BC preserved vaccine stored for 18 weeks at 37°C showed no measurable mouse-protective potency (Table 4). However, the impairing effect of BC treatment could be reduced by the treatment of pertussis vaccine with aluminium, calcium or magnesium salts or by choline or D-lysine before addition of the BC. It was suggested that these substances are capable of preventing the absorption of BC onto the pertussis cells, thus stabilizing the protective antigens as measured by the mouse protection test.
### Table 4. Potency of pertussis vaccines containing various preservatives and stored at 37°C

According to Olson et al. (83)

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Potency in IU/ml</th>
<th>Number of weeks storage at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Merthiolate</td>
<td></td>
<td>4.6</td>
</tr>
<tr>
<td>Benzethonium chloride BC</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>BC + calcium chloride</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>BC + aluminium phosph.</td>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td>BC + magnesium sulfate</td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td>Choline</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

NP = no protection found

Van Ramshorst and van Wezel (92) studied the stability of all components of quadruple DPT polio vaccines, preserved with BC and 2-phenoxyethanol and formaldehyde. The potency loss rates of the pertussis component of the vaccines preserved with BC and phenoxyethanol were not essentially different from that of merthiolate-preserved vaccine. It is possible that aluminium phosphate present in the Dutch quadruple vaccine reduced the deleterious effect of benzethonium chloride.
4. BCG VACCINE

The standardization of and studies on the stability of BCG vaccine are complicated by several factors including:

1. the number of different substrains (daughter strains) used in vaccine production;

2. differences in manufacturing and testing procedures employed by vaccine producers;

3. the different bacterial content and different number of culturable particles in different products; and

4. the lack of an approved laboratory method of assaying the protective potency of vaccines against tuberculous infection in man.

For batch-to-batch quality control, the most important test is the checking of vaccine viability by determining the number of culturable particles (CP) by means of colony counts on solid medium. The viability test is also of prime importance in assessing the stability of BCG stored at different conditions.

BCG is the first EPI vaccine for which a WHO requirement for heat stability was established (110). Each lot of BCG vaccine should be tested for heat stability in an accelerated degradation test. The number of CP in vaccine incubated at 37°C for 28 days should be not less than 20% of that in the vaccine stored at 4°C (113).

4.1. Impact of temperature on the viability of BCG vaccine

BCG vaccine is relatively stable at refrigerator temperatures below 8°C. The annual loss of viability at this temperature may be estimated as approximately 1% (38, 120) judging from the fact that during two year's storage at 4°C, the viability decreased by about 20% (38). However, it has been reported that some vaccines can lose as much as 20% to 25% of their original viability after only 6 months storage (102). Most of manufacturers give a one year validity period for storage at refrigerator temperatures of below 8°C.

BCG vaccines showed only slight loss of viability when kept at 13°C - 15°C for two months but the loss of viability reached about 20% at the end of 9 months of storage (18). The viability was reduced by approximately 10% per month by exposure to 18°C (120).
At room temperature (22°C -25°C), some BCG vaccines may lose one fifth to one third of their original viability after 3 months storage (18). At higher temperatures (30°C or 37°C), degradation starts off very rapidly and the rate of the CP reduction is greater at the beginning than during later stages of exposure (Table 5) and Sekhuis et al. (96). It is not known whether this early degradation rate would be as steep if exposure to high temperatures is repeated.

Table 5. Loss of viability of four BCG vaccines stored at 30°C and 37°C for 36 weeks
Based on data from Bunch-Christensen (18)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Rates of potency loss per day in % of original value at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°C</td>
</tr>
<tr>
<td>storage period in weeks</td>
<td>0 - 9</td>
</tr>
<tr>
<td>Japanese</td>
<td>0.5</td>
</tr>
<tr>
<td>Glaxo</td>
<td>0.9</td>
</tr>
<tr>
<td>Dakar</td>
<td>1.0</td>
</tr>
<tr>
<td>Danish</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Daily loss of the viability of vaccines kept for a few weeks at 37°C ranged from 1% to 2% (11, 16, 18, 120).

At temperatures higher than 37°C, the degradation of BCG vaccine is very rapid. The viability may be reduced by half after exposure to 70°C for 30 minutes or by 80% after 5 minutes' boiling (42).

The exposure of BCG vaccine to elevated temperatures clearly results in reduction of the number of CP which is proportional to the height of temperatures and the length of exposure. However, since the optimal dose of the vaccine is not known it is difficult to determine a permissible limit of vaccine
heat degradation. Results of some studies suggest that vaccines with viability reduced to 40% -60% following 2 to 4 weeks exposure to elevated temperatures were still able, when tested in school children, to give tuberculin sensitivity and vaccination lesions which were indistinguishable from those given by refrigerator-stored control vaccines (16, 120). Longer storage at elevated temperatures affected BCG vaccine by reducing post vaccination allergy and size of vaccination lesion (16). The interpretation of these results is not easy since delayed type hypersensitivity to tuberculin and local vaccination granulomas, the hallmarks of specific cellular responses, are not directly related to protection.

Questions are often raised whether BCG vaccine may be stored below 0°C. One may speculate that repeated freezing and thawing could be deleterious to the vaccine owing to the recrystalization of the residual moisture. The experimental data do not support that speculation. Storage at -20°C or -30°C, or repeating freezing and thawing cycles (up to 10 times) do not appear to affect the viability of BCG vaccines (18, 42).

Table 6. Viability and heat stability of 10 BCG vaccines
According to (63)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Initial no. number of CP 10^6/ml</th>
<th>Viability after storage for 28 days at 37°C CP x 10^6/ml</th>
<th>% of initial</th>
<th>Daily loss of viability in % (storage period analysed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese</td>
<td>27.0</td>
<td>16.6</td>
<td>61</td>
<td>1.5 (0 - 28)</td>
</tr>
<tr>
<td>Glaxo</td>
<td>20.1</td>
<td>10.9</td>
<td>54</td>
<td>1.9 (0 - 21)</td>
</tr>
<tr>
<td>USSR</td>
<td>7.1</td>
<td>3.6</td>
<td>51</td>
<td>1.8 (0 - 28)</td>
</tr>
<tr>
<td>Connaught</td>
<td>6.9</td>
<td>0.2</td>
<td>3</td>
<td>6.7 (0 - 14)</td>
</tr>
<tr>
<td>Dakar</td>
<td>6.5</td>
<td>1.8</td>
<td>28</td>
<td>3.2 (0 - 21)</td>
</tr>
<tr>
<td>Bilthoven</td>
<td>4.2</td>
<td>1.3</td>
<td>31</td>
<td>4.9 (0 - 14)</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>2.9</td>
<td>1.9</td>
<td>66</td>
<td>2.5 (0 - 28)</td>
</tr>
<tr>
<td>Merieux</td>
<td>2.8</td>
<td>0.3</td>
<td>11</td>
<td>3.3 (0 - 28)</td>
</tr>
<tr>
<td>Pasteur Inst</td>
<td>2.7</td>
<td>1.3</td>
<td>48</td>
<td>1.9 (0 - 28)</td>
</tr>
<tr>
<td>Prague</td>
<td>1.1</td>
<td>0.2</td>
<td>18</td>
<td>5.2 (0 - 21)</td>
</tr>
</tbody>
</table>

4.2. Stability of vaccines produced from different BCG substrains

All available BCG strains distributed throughout the world are derived from the same strain produced by Calmette more than 65 years ago. The long period of maintenance by culture medium transfers of the original strain resulted in essential differences in daughter strains.
BCG strains are usually classified as "strong" such as the French strain 1173-P2 (Pasteur) or Danish strain 1331 (Copenhagen), or "weak" strains such as the Japanese strain 172, the Brazilian strain Moreau or the British strain 1077 (Glaxo). This distinction is based mainly on growth characteristics, residual virulence in animals and reactogenicity in children. Recent studies provide some evidence that these differences may be linked with surface antigenic lipid content and protein secreted by these strains (1).

There are differences in the heat stability of BCG vaccine prepared from different substrains (Figure 6). At all temperatures tested, the degradation rates were lowest for Japanese vaccine and highest for Dakar and Glaxo vaccines, with Danish vaccine occupying an intermediate position. During longer storage, the differences between vaccines declined (Table 5). Other studies also showed differences between the stability of different BCG vaccines (Table 6, and Gheorghiu and Lagrange (41), Sekhuis et al. (96).

**Figure 6.**
Viability of four BCG vaccines stored for 36 weeks at various temperatures according to (18)

4.3. Packing BCG vaccines

BCG vaccine requires special precautions in order to ensure sufficient stability. In this respect the most important measures are lyophilization, an effective stabilizer, and proper sealing of the vaccine container.
Better stability at 4°C and 37°C and higher starting viability values (i.e. better survival rates after freeze drying) were observed after changing the composition of the stabilizer and improvement of the drying method (38).

At present the use of ampoules sealed under vacuum is the most common practice. While vacuum-sealing adds to the stability of vaccine, it involves a difficult manufacturing process; sealing in the presence of inert gas is simpler. There were no significant differences between BCG vaccines sealed under vacuum and under nitrogen or carbon dioxide, either at 4°C or 37°C (38, 60). In another study, however, the viable counts for the vaccine sealed under nitrogen declined more rapidly than for that sealed under vacuum (16). A BCG vaccine sealed under argon seemed to have worse stability at 37°C than vaccine sealed under vacuum (42).

It was found that BCG vaccines in rubber stoppered vials had a lower stability than those conserved in ampoules (63, 96). A further disadvantage of rubber stopped vials is that the users are tempted to keep the reconstituted vaccine (104).

4.4. Effect of light on BCG vaccine stability

Freeze dried BCG vaccines, regardless of the substrain used for production, are sensitive to ultraviolet and fluorescent light and should be packed in ampoules of low light transmittance (such as amber glass) and protected from light when used (61).

4.5. Stability of reconstituted vaccine

Reconstituted BCG vaccine is very unstable and should be used promptly during one working session (5 to 6 hours). The residual vaccine should be discarded at the end of the session. These precautions are based on two concerns:

1. the risk of contamination, because BCG vaccine, in contrast to all other vaccines does not contain any bacteriostatic agent and

2. the loss of potency (31).
5. MEASLES, MUMPS AND RUBELLA VACCINES

5.1. Measles vaccine

5.1.1. 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 (43, 72, 87) and the formulation of a WHO requirement for heat stability for freeze dried measles vaccine (112 and Figure 7), have made a considerable impact on the quality of measles vaccines now available on the market.

The "first generation" vaccines exposed for one week to 37°C lost from 0.7 log_{10} to 2 log_{10} units (5, 21, 23, 43, 75, 87). In 1979, the results of a comparative study of the heat stability of measles vaccines from different manufacturers showed that out of 6 manufacturers, only two produced vaccines with enhanced resistance to heat (30). Most of the vaccines dropped below minimum potency (1 000 TCID_{50} of PFU per human dose) after only few days at 37°C (33).

**Figure 7**
WHO requirements for the stability of freeze-dried measles vaccine
According to (112)

The thermal degradation of the "second generation" measles vaccines is much slower (Figure 8, and 4, 70). At 2°C to 8°C, these improved vaccines maintain minimum potency for more than two years (6, 72).
In the process of degradation of measles vaccine at elevated temperatures two components can be distinguished:

-the first component, a rapid loss of titer that seems to be accentuated at high temperatures but appears to be inoperative below 20°C, and
-the second component with a slower degradation rate (4).

Second generation vaccines can be expected to lose less than 0.3 \( \log_{10} \) infectivity titer during a one week exposure to 37°C, when taking into consideration both components (Table 7). The rate of degradation due to the second component is low and does not exceed 0.1 \( \log_{10} \) per week (23).

At 41°C, the degradation proceeds quickly with the titer decreasing by 50% within two days and by 0.4 - 0.7 \( \log_{10} \) within one week (36, 87). The half life of second generation measles vaccine (i.e. the period during which the vaccine loses half of its original titer) is estimated to be 31 days, 16.6 days and 3.3 days at 20 - 25°C, 37°C and 41°C, respectively (6).

**Figure 8**

Results of accelerated stability tests at three temperatures for a first generation vaccine (a) and a second generation vaccine (b). Value for unexposed vaccines indicated by a star. According to (4 and 70).

At 45°C the potency of the AIK-C strain vaccine declined at a rate of 0.22 \( \log_{10} \) TCID\(_{50}\) per day so that after one week of storage at this temperature the titer of the vaccine dropped by 1.6 \( \log_{10} \) TCID\(_{50}\) (66).

At 54°C to 56°C, measles vaccine is inactivated rapidly, losing more than 0.65 \( \log_{10} \) and 1 \( \log_{10} \) during one and three day exposures (72).
Table 7. Loss of infectivity titer in log_{10} per week in freeze-dried measles vaccines stored at 37°C
According to (23)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>loss of infectivity in log_{10} per week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>both components</td>
</tr>
<tr>
<td>A</td>
<td>0.25</td>
</tr>
<tr>
<td>B</td>
<td>0.23</td>
</tr>
<tr>
<td>C</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The enhanced resistance of the second generation measles vaccines has been confirmed in the field. Two freeze-dried vaccines stored for 7 days at 37°C maintained both the minimum infectivity titer and the ability to induce seroconversion in all 9 - 24 month aged children tested (47).

However, there are still differences in stability of measles vaccines available on the market. From Figure 9 one can see that although four vaccines produced from different virus strains fulfill the WHO requirements for stability, only one vaccine has long-lasting resistance to elevated temperature and can be called a third generation vaccine. Titers of the remaining three vaccines declined below the minimum potency level following 10 to 12 day storage at 37°C.

Figure 9

Titers of measles virus in four measles vaccines prepared from different strains and stored at 37°C
Schwarz and L-16 strain vaccines – according to (36), Edmonston-Zagreb strain vaccine – according to (67) and Moraten strain vaccine – according to (72)
Measles vaccines prepared from AIK-C and CAM-70 strains, showed considerable differences in stability. The AIK-C vaccine has been stabilized with a stabilizer containing 2% hydrolyzed gelatin, 5% lactose, 5% sucrose, 1.8% d-sorbitol and 0.1% sodium glutamate in 199 medium. The potency of this vaccine declined by $0.5 \log_{10} TCID_{50}$ during 2 weeks and by $1.1 \log_{10} TCID_{50}$ during a 4 week storage at $37^\circ C$ (66). The average degradation rate was some $0.04 \log_{10}$ per day at $37^\circ C$.

The CAM-70 vaccine showed a more rapid degradation at $37^\circ C$. It lost $0.76 \log_{10} TCID_{50}$ following one week storage (103) and the average degradation rate was $0.11 \log_{10} TCID_{50}$ per day.

The differences observed between these two vaccines cannot be explained simply by the different strains of measles virus used for their production. Other AIK-C vaccine prepared by a different manufacturer seemed to have a heat stability similar to that of CAM-70 vaccine and lost $0.1 \log_{10} TCID_{50}$ per day at $37^\circ C$ (79).

These findings suggest that the differences in heat stability among measles vaccines are not related to the various measles virus strains but rather to different stabilizers added before lyophilization.

5.1.2. Reconstituted vaccine

The improved stability of measles vaccines refers to vaccines in the freeze dried state. Measles vaccines, even those of the "second generation", quickly lose their potency when reconstituted and kept at elevated temperatures.

Reconstituted measles vaccines kept at $4^\circ C$ retained their potency above 1000 TCID$_{50}$ for at least 24 hours, although they showed a slow degradation at the rate of $0.015 \log_{10} TCID_{50}$ per hour (36). The storage of these vaccines for one hour at room temperature or at $37^\circ C$ resulted in some 50% loss of titer (Table 8). A six hour exposure to $37^\circ C$ results in a 10-fold decline in virus titer. A vaccine reconstituted with diluent prewarmed to $41^\circ C$ and incubated further at that temperature losses 0.3 and $1 \log_{10}$ of its titer after half an hour and 3 hours respectively (Table 8). The virus strain from which the vaccine is prepared and the nature of diluent used for reconstitution appear not to have a significant influence on stability.

Field experience shows the danger of storing reconstituted vaccine at ambient temperature; among seronegative children vaccinated with reconstituted vaccine stored at $37^\circ C$ for 4 hours 92% seroconverted, while among those vaccinated with reconstituted vaccine stored for six hours at that temperature only 78% seroconverted (47).

5.2 Mumps vaccine

Both components of a bivalent lyophilised measles-mumps vaccine have a similar stability at $4^\circ C$, $23^\circ C$, $37^\circ C$ and $45^\circ C$. At $37^\circ C$, the degradation rates were about $0.01 \log_{10}$ per day for both components. Half lives were also similar: 4.7 and 5.4 days for measles and mumps components at $45^\circ C$, 12 days and 13 days at $37^\circ C$ and 71 days and 65 days at $23^\circ C$ (24).
Table 8. Heat stability of measles vaccines reconstituted with different diluents and exposed to elevated temperatures

Schwarz strain vaccines - according to (87 and 36); Moraten strain vaccine - according to (21); L-16 strain vaccine - according to (36) and Edmonston-Zagreb strain vaccine - according to (49).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Virus vaccine strain</th>
<th>Reconstituted in:</th>
<th>loss of titer in log₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Schwarz¹</td>
<td>Moraten²</td>
<td>Schwarz³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>0.29</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.72</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.23</td>
<td>0.2</td>
<td>0.33</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>0.41</td>
<td>0.45</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>0.45</td>
<td>0.36</td>
<td>-</td>
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<tr>
<td>4</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>5</td>
<td>-</td>
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<tr>
<td>6</td>
<td>-</td>
<td>-</td>
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<tr>
<td>7</td>
<td>-</td>
<td>-</td>
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<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>24</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
The mumps vaccine component in mumps-rubella vaccine and in measles-mumps-rubella (MMR) vaccine shows degradation rates comparable to those for monovalent mumps vaccine at 20°C to 56°C (72).

5.3. Rubella vaccine

Freeze dried monovalent rubella vaccine as well as the rubella component of bivalent vaccines (measles-rubella or mumps-rubella) or of trivalent MMR vaccine show low degradation rates. At 37°C the average loss of titer ranged from 0.046 to 0.109 log_{10} TCID_{50} per week exposure. In comparison with other components of combined virus vaccines, the rubella component seems to be the most stable (72).
6. POLIOMYELITIS VACCINE

6.1. Oral poliomyelitis vaccine

Oral poliomyelitis vaccine (OPV) is the least heat stable of the EPI vaccines. Recently developed technologies have resulted in considerable enlargement of knowledge of genetic and structural aspects of poliovirus (55). Progress in basic knowledge has not yet resulted in improvements of the stability of OPV. Presently available stabilizers, such as some sugars and magnesium chloride, are not yet sufficient and more research is needed to improve the heat resistance of OPV.

More information is needed on the freezing point of OPV. The presence of 1 molar magnesium chloride (MgCl₂) as a stabilizer in vaccine preparations lowers the freezing point of vaccine virus suspensions to -11°C. Freezer compartments of refrigerators, sometimes used for storage of OPV, operate at temperatures of about -5°C. This is above the melting point of the vaccine, and does not guarantee that the vaccine remains solid.

In a freezer with temperature normally below -11°C repeated defrosting (especially in models which have automatic defrosting) may subject the vaccine virus to freeze-thaw cycles. Some studies have shown that there is no significant loss of the virus titer of OPV subjected to up to 10 cycles of freezing and thawing (36, 37, 64, 98). However in these reports no details are given concerning the rapidity of freezing and thawing, the temperature to which samples were raised during each thawing or the length of intervals for which the vaccine was kept thawed. In these studies the total titers were measured for trivalent vaccine and no data are available for type specific polio virus sensitivity to freeze-thaw cycles. All these factors may influence the survival of virus particles during freezing-thawing cycles (10).

6.1.1. Factors affecting the stability of OPV

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

Differences in heat sensitivity of the viral types

In most immunization programmes trivalent OPV is used. The individual poliovirus types in the triple vaccine differ in their growth rates. Type II has the most prolific growth during intestinal replication, followed by types III and I. To compensate for these differences in growth rate, trivalent vaccine should contain types I, II and III in proportions of 10:1:3 (114). Studies are continuing on alternative formulations of OPV (84).
If the heat sensitivity of the three virus types differs, then partial heat inactivation during a cold chain break may change the balance of the vaccine in favour of resistant type(s) and interfere with replication of the remaining type(s).

Mauler and Gruschkau (71) draw attention to the possibility of differences in heat stability between poliovirus types. Results of testing 50 commercial lots of OPV stored at 20°C to 60°C suggested that type II is particularly stable, whereas type I is the least stable. The same differences in stability were found when vaccines were stored at 250°C (Figure 10).

These observations have not been confirmed by other authors who tested different OPV vaccines. According to Peetermans et al. (86), type I has been more stable than types II and III. Type I showed only 0.06 log10 loss after 12 months storage of OPV vaccine at 40°C compared with 0.20 and 0.27 log10 losses, respectively, for types II and III. The differences between various types are not consistent and there was no clear evidence of higher resistance of a particular virus type when vaccines were stored at 20°C to 25°C and at 37°C.

**Figure 10**

Stability of the trivalent oral poliovirus vaccine stabilized with buffer-peptone and stored at 20°C to 60°C and at 25°C

According to (70)

---

A. Storage at 2 to 6°C

B. Storage at 25°C
(Table 9). Mirchamsy et al. could not find differences between poliovirus types kept for 9 months at +4° C and at -20° C (79).

Table 9. Comparison of the stability of different poliovirus types exposed to the temperature of 20° C - 25° C and 37° C

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Loss of titer in log TCID₅₀ per day at temperature of:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20° C - 25° C</td>
<td>37° C</td>
</tr>
<tr>
<td></td>
<td>Type I</td>
<td>II</td>
</tr>
<tr>
<td>MgCl₂-stabilized</td>
<td>0.026</td>
<td>0.024</td>
</tr>
<tr>
<td>sucrose-stabilized</td>
<td>0.043</td>
<td>0.064</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
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</tr>
</tbody>
</table>

Nature of stabilizer

Various substances have been used for stabilization of attenuated polioviruses: magnesium chloride, saccharose, buffers, milk and gelatin. The first two stabilizers have been used most frequently.

Wallis and Melnick (107) reported that polioviruses were stabilized by the addition of cations to the suspending medium. In particular, the addition of 1 molar magnesium chloride (MgCl₂) to attenuated poliovirus strains enabled vaccines to be stored at 4° C for 3 months or at 25° C for 25 days without significant loss of titer. In another study, Melnick et al. (73) found that MgCl₂-stabilized vaccines 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 feeding.

Other studies showed that 35% - 50% sucrose was also effective in stabilizing attenuated polioviruses. Perkins and Magrath (90) and Magrath (65) concluded that both 1 M MgCl₂ and 50% sucrose are effective stabilizing agents, but in order to achieve maximum virus stability it is necessary to prevent the rise in pH that occurs with the loss of CO₂ from the container.
Both stabilizers are used by various manufacturers. At a symposium on the stability of vaccine in Zagreb in 1976, it was concluded that sucrose appeared to be as effective as MgCl₂ in some vaccines. The greater acceptability of sucrose to children due to its sweetness, in contrast to the bitter taste of MgCl₂, was also emphasized.

At present, most oral polio vaccines available on the market are stabilized with magnesium chloride, although some manufacturers produce sucrose-stabilized poliovaccines. More recent studies appear to show a higher efficacy of magnesium chloride than sucrose in increasing thermostability of OPV. The rate of potency loss in monovalent vaccines of all three types was higher with saccharose or buffer stabilized vaccines than with magnesium chloride vaccines when kept for 8 days at 37°C or 29 days at 25°C (Figure 11).

Data on stability on two vaccines from one manufacturer showed that magnesium chloride stabilized vaccine is more stable than the sucrose stabilized vaccine at temperatures below 37°C (Table 10). The better stabilizing effect of magnesium chloride was also observed by another producer (79).

The workshop on Temperature Stable Vaccines held in Washington in April 1987 concluded that the consistent use of magnesium chloride would help in increasing the stability of OPV and in minimizing reliance on the cold chain (50). Buffered sucrose with carefully controlled pH may also be an efficient stabilizer, however, since pH plays an important role in poliovirus vaccine stabilization (see below).

Poliovirus can be also stabilized against heat inactivation by adding fatty acids and related compounds. Incubation of type I Sabin poliovirus with myristic acid for 30 minutes at 45°C resulted in 19% reduction of infectivity, while incubation of poliovirus without this fatty acid resulted in 99% loss of infectivity (28). Thermal stabilization was also observed when poliovirus was incubated with other fatty acids: hexanoic, octanoic or palmitic acids. The presence of these stabilizers during heating may prevent conformational changes in the capsid that render the virus non-infectious.

pH values of virus suspensions

Melnick and Wallis showed in 1963 the importance of pH values in maintaining the stability of OPV (74). They demonstrated that pH values increased in all tested vaccines, but these increases were much more marked in loosely capped vials than in those which were tightly stoppered. Vaccines with 6.0 - 6.4 initial pH in tightly stoppered vials manifested no significant loss of infectivity after 20 days at temperatures of 25°C to 28°C; the titer dropped at the end of the observation only 0.1 - 0.2 log₁₀ (Table 11). The samples in loosely capped vials lost their infectivity rapidly, evidently due to an increase in pH.
Figure 11
Loss of potency of monovalent OPV stabilized with magnesium chloride, buffer and sucrose and stored at 37°C and 25°C. b values mean loss of titer per day (regression coefficients for regression lines) According to (70)

Similar observations were made by Mauler and Gruschkau (71) who studied monovalent poliovirus vaccines with pH values ranging from 5.5 to 8.0. After 3 days at 37°C, the highest losses were found in extreme pH ranges, 5.5 and 8.0 (Figure 12). Poliovirus vaccines were remarkably stable within the pH range of 6.5 to 7.2.

Vaccines can be maintained in this pH range by preventing the loss of carbon dioxide from a bicarbonate-containing vaccine, keeping the air space above vaccine to a minimum, and packing the vaccine in tightly stoppered containers (8).
Table 10. Average losses of the total virus content and half life of oral polio vaccines stabilized with sucrose and magnesium chloride and stored at various temperatures.

According to (88)

<table>
<thead>
<tr>
<th>Storage temperature in °C</th>
<th>Time unit</th>
<th>sucrose</th>
<th>magnesium chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>titre loss*</td>
<td>half life*</td>
</tr>
<tr>
<td>4</td>
<td>month</td>
<td>0.11</td>
<td>6</td>
</tr>
<tr>
<td>20 - 25</td>
<td>day</td>
<td>0.03</td>
<td>12</td>
</tr>
<tr>
<td>37</td>
<td>day</td>
<td>0.15</td>
<td>1.9</td>
</tr>
<tr>
<td>45</td>
<td>day</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*per in time unit showed

Table 11. Values of pH and the decrease of titer of MgCl₂-stabilized type I OPV stored for 20 days at 25°C - 28°C

According to (74)

<table>
<thead>
<tr>
<th>Vaccine packed in container</th>
<th>pH values/log10 decrease in infectivity titer (PFU/ml) **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial pH*</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Tightly stoppered</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Loosely stoppered</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*Type I oral polio vaccine was mixed with an equal volume of 2 M MgCl₂ and pH levels were adjusted as shown above. After 1, 10 and 20 days, pH levels were again tested.

**Vaccines diluted to contain initially 10⁷ PFU/ml
Containers in which the vaccine is dispensed

Glass has been considered to be the best material for vaccine containers (8). Out of two vaccines stabilized with MgCl₂, that packed in glass bottles and sealed with a rubber wad appeared to be more stable than the other vaccine dispensed in 5 ml polythene bottles. The third vaccine packed in nylon containers and sealed with a screw cap had intermediate stability (Table 12).

**Figure 12**
Effect of different pH values on stability of attenuated polioviruses at 37°C for three days
According to (71)

![Graph showing pH values and loss of titer in log TCID₅₀/ml](image-url)

Recently, a manufacturer has prepared polio vaccines in low density polyethylene tubes with an easy to use dispenser for mass immunization. The stability tests were performed with monovalent vaccines packed in glass and polyethylene containers and stored for 14 days at 26°C and for 8 months at 50°C. There was no clear difference in the stability of oral polio vaccines packed in glass vials and plastic tubes (Table 13).

6.1.2. Overall stability of polio vaccines

The stability of trivalent polio vaccine has usually been monitored by measuring the total content of live viruses of the three serotypes (17, 36, 79, 86, 98). This practice favours the type I content and may overlook changes in the type II and type III content following exposure to elevated temperatures (10).
Since there is a close relationship between the storage temperature and the poliovirus survival, the manufacturers recommend different expiry dates for OPV kept at different temperatures. Most manufacturers include two storage temperatures: (1) a period up to 2 years when stored in a deep freezer at or below -20°C, and (2) a period of 6 to 12 months when stored in a refrigerator at temperatures of 0°C to 8°C. Health workers are experiencing some difficulties in responding to the different expiry dates. It can happen that a vaccine stored for some months in a freezer followed by months storage in refrigerator may still be used based on the expiry date shown on the pack for the freezer storage.

Table 12. The influence of container on the stability of oral poliovaccines
According to (65)

<table>
<thead>
<tr>
<th>Container</th>
<th>Sealing</th>
<th>Vaccine volume (ml)</th>
<th>Dose per vial</th>
<th>Stabilizer</th>
<th>Log10 loss in titer at temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ml glass bottles</td>
<td>rubber wad</td>
<td>1.5</td>
<td>10</td>
<td>MgCl2</td>
<td>4-10°C: 0.02/week, 21°C: 0.4/month</td>
</tr>
<tr>
<td>5 ml polythene bottles</td>
<td>plastic screw cap</td>
<td>1.5</td>
<td>10</td>
<td>MgCl2</td>
<td>0.74 per month</td>
</tr>
<tr>
<td>1.5 ml nylon containers</td>
<td>screw cap</td>
<td>1.0</td>
<td>10</td>
<td>50% sucrose</td>
<td>0.04 per week</td>
</tr>
</tbody>
</table>

When the distribution and administration of OPV is not imminent, the EPI recommends its storage at temperature below -15°C for up to 8 months at central level and for up to 3 months at regional level. At these levels reliable freezers are usually available. In the field, where the chance of a serious cold chain break is high and where freezers are less available, OPV should be kept at the health center level for as short a time as possible (up to one month) at refrigerator temperatures (0°C to 8°C). Transport of the vaccine can also be done at refrigerator temperature but for a period not longer than one week (35, 62).
Table 13. Loss of titer of monovalent poliovirus vaccines packed in glass vials and plastic tubes and stored at 50°C for 8 months and at 260°C for 14 days. According to direct information from producer (95)

<table>
<thead>
<tr>
<th>Duration and temperature of container</th>
<th>Type of container</th>
<th>Type of virus</th>
<th>Loss of titer per time unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>in %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 months at 50°C</td>
<td>Glass vials</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Plastic tubes</td>
<td>1</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>7.4</td>
</tr>
<tr>
<td>per day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 days at 260°C</td>
<td>Glass vials</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Plastic tubes</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.9</td>
</tr>
</tbody>
</table>

A single expiry date would be optimal to avoid problems with the storage of OPV. The Biologicals Unit of the WHO/HQ is currently working to resolve this problem in the cooperation with manufacturers.

Results of the accelerated degradation test suggest that oral poliovirus vaccines retain their initial titers for 7 to 14 days at 260°C, and for 2 days at 310°C (36).

There is about a 10-fold difference in the average titer loss per day when a vaccine is exposed to temperatures of 220°C to 260°C as compared with 370°C. The estimated titer losses were 0.01 - 0.04 log10 and 0.10 - 0.20 log10, respectively (36, 72, 88, 98).
With these degradation rates, a vaccine with a total virus content of 6.15 log_{10} will lose half of its potency during a two day exposure to 37°C or during a 20 day exposure to 22°C to 26°C. This corresponds well with the early observations of Melnick and Wallis (74) and Perkins and Magrath (90), who considered that poliovirus vaccines retain minimum potency for 3 days at 37°C and for 14 to 21 days at 25°C to 28°C.

Mann et al., (68), however, recently studied 10 commercial trivalent poliovirus vaccines, and observed shorter half lives, ranging from 1 to 2 days at 37°C and from 5 to 11 days at 25°C.

At temperatures over 37°C, the degradation of poliovaccine occurs rapidly. At 41°C vaccines lose about half of their potency each day (36), while at 50°C a vaccine loses 0.1 log_{10} during one hour (17). The half life of such a vaccine would then be 3 hours.

The Biologicals Unit at the WHO/HQ is working to establish a WHO requirement for thermostability of poliovirus vaccines (116). Such a requirement could have a positive effect on poliovirus vaccine stability, similar to the effect seen after the measles vaccine requirement was established in 1981.

6.2. Inactivated poliovirus vaccine (IPV)

Certain treatments are known to destroy the capacity of poliovirus to produce neutralizing antibodies. Three of these are heat, freeze-drying and addition of merthiolate (thiomersal). As mentioned in paragraph 3.6, the poliovirus component of a quadruple DPT Polio vaccine was not stable when merthiolate was used as a preservative. Beale & Ungar (15) demonstrated a rapid fall in potency of the polio antigen in a merthiolate- and sodium edetate-preserved quadruple vaccine stored at 4°C. Another lot of quadruple vaccine without merthiolate but with half the amount of sodium edetate was stable over one year.

It appears that there are some differences in heat stability of various inactivated poliovirus types, type I being most vulnerable. In the absence of any preservative, the type I component of trivalent poliovirus vaccine deteriorates slowly after two years of storage at 4°C, while the two remaining types remain potent for 20 years. The D-antigen content for type I dropped significantly after 20 days at 24°C and was undetectable after exposure to 32°C for the same period. No significant changes in D-antigen value were observed for type II antigen at either of these temperatures, while type III remained stable for 20 days at 24°C, but dropped significantly at 32°C (80).
All three types of IPV showed satisfactory retention of potency when incorporated into combined vaccines and stored at 40°C for one to over 4 years. These observations were made with aluminium hydroxide adsorbed DíTePolio vaccine preserved with benzethonium chloride (80) and with aluminium phosphate DPT-Polio vaccine without any preservative or with phenoxyethanol and formaldehyde as preservatives (92). Longer storage resulted in a decline in antigenicity, especially of the type I component (80).

At 37°C, the D-antigen content of the poliomyelitis component of a quadruple vaccine decreases during storage, but most of the potency is left after 8 weeks. Type III seems to be most stable component (92).
7. STABILITY OF OTHER VACCINES

7.1. Viral vaccines

7.1.1. Hepatitis B vaccine: Two types of vaccines are available on the market: a plasma derived vaccine and a recombinant vaccine. Both are adsorbed on aluminium salts and, as with other adjuvanted vaccines, should be protected from being frozen. Their freezing point is about -0.5°C.

At temperatures 20°C to 80°C, hepatitis B vaccine appears to be stable for many years. The upper limits of storage life have not been defined.

A yeast derived hepatitis B vaccine (Engerix-B, Smith Kline Biologicals) seems to be a relatively stable product at elevated temperatures. The manufacturer considers that the vaccine is stable for 30 days at 20°C - 25°C, for one week at 37°C and for 3 days at 45°C. The calculated half life at these three temperatures was 9 months, 31 days and 13 days (direct information from the manufacturer).

There were no differences in the immune responses of healthy persons immunized with a recombinant vaccine heated to 37°C for one week and a control vaccine stored at 4°C; the antibody distribution and geometric mean titers of antibody were similar in the two groups. The total incidence, severity and types of symptoms were similar in persons immunized with the two vaccines; no severe reactions were reported (53).

A vaccine from another manufacturer can withstand temperatures over 30°C for no more than 5 hours (77).

More information on comparative stability for different hepatitis B vaccines is needed.

7.1.2. Yellow fever vaccine (YF)

The poor stability of the early yellow fever vaccines has been a matter of concern. Most of these vaccines lost some activity during a 6 month period of storage at -20°C or 50°C, and they deteriorated quite quickly when held in higher temperatures (93a). Research undertaken in a number of laboratories revealed that stabilizing media such as lactose, sorbitol, histidine and alanine considerably improve the heat stability of lyophilized 17D vaccine (12, 13) and that stabilized vaccines may be successfully used in different field conditions (39, 94). The development of a more stable formulation of YF vaccine provided a product whose shelf life at a temperature of -20°C or 40°C can be prolonged to 2 years. The estimated half life of the infectivity of the vaccine is about 10 months at room temperature, from 10 to 20 days at 37°C and about 2 days at 46°C (19, 37). The difference between the stability of vaccine of the old and new formulation is shown in Figure 13. The stabilized vaccine shows a similar titer decline when held continuously at 37°C and when subjected to alternative cycles of 37°C and 40°C (37).
However, vaccines available at present on the market differ considerably in their stability. A collaborative study undertaken with the cooperation of the 12 manufacturers of the 17D YF vaccines (108) showed a very wide range of stability among vaccines stored at 37°C for 32 days (Figure 14). The results of the study indicated that some vaccines still contained at least 1 000 PFU after 14 days exposure while other vaccines lost most of their activity after 1 to 5 days exposure.

The WHO has established a heat stability requirement for YF vaccine: it should retain 1000 mouse LD₅₀, or its equivalent in plaque forming units per human dose and the mean loss in titer should be less than 1 log after being heated for 2 weeks at 37°C (115). It is hoped that the stability requirement will, as in the case of measles vaccine, stimulate the production of more stable YF vaccines.

Figure 13
Decrease of infectivity of lyophilized, stabilized and non-stabilized yellow fever virus vaccines kept at 46°C for 4 days
According to (19)

After reconstitution, the stabilized vaccine loses its resistance to heat. When kept in an ice-bath at an ambient temperature of 37°C the vaccine may be fairly stable for up to 3 hours (about 5% loss per hour) but dilution with a diluent of 37°C resulted in rapid virus inactivation with total loss of activity within one hour (99). Another stabilized YF vaccine lost about half of its infectivity after some 90 minutes at 36°C and after 45 minutes at 46°C (19).
Figure 14
Rating of 17D yellow fever vaccines according to the proposed requirements of heat stability
According to (108)

Of various diluents, including distilled water, saline, buffer, gelatin and peptone, distilled water was the most effective in maintaining a satisfactory virus titer of vaccine for 3 hours after reconstitution at 37°C (99).
7.1.3. Rabies vaccine

Every year many doses of Semple and other neural tissue rabies vaccines are distributed in developing countries for rabies post exposure prophylaxis. The Semple vaccine may cause paralytic and other adverse reactions and in its fluid state has a short shelf life (several months) at 4°C (2).

The suckling mouse brain (SEM) vaccine widely used in Latin America is more stable than Semple vaccine. When stored at 4°C for one year, the vaccine retains minimum acceptable potency, although the estimated average potency loss is about 42%. Storage at 25°C for one month did not cause deterioration below the minimum level of potency but the vaccine lost potency following storage for less than one months at 37°C (27). Lyophilized SEM vaccines are more stable than liquid ones, but a critical factor for their stability is the residual moisture (27).

Presently recommended lyophilized human diploid cell strain rabies vaccine (HDCSV) stored at 4°C has a much longer shelf life than neural tissue vaccines and can withstand storage at 37°C for one month without loss of potency in mice (82). HDCSV vaccine dispatched, transported and stored at temperatures from 26°C to 36°C for up to 11 weeks has stimulated similar antibody responses in Pakistani medical health staff volunteers as the vaccines transported and stored at 20°C to 13°C (81).

7.2. Bacterial vaccines

7.2.1. Meningococcal polysaccharides

The immunogenicity of meningococcal vaccine is related to the molecular size of the protective antigens - polysaccharides A and C. The higher the molecular weight the higher the antibody response. Polysaccharide antigens readily depolymerize and their relative molecular mass diminishes when they are exposed to ambient temperatures. The study of the degree of polymerization is therefore useful for assessing both immunogenicity and thermal stability.

Lactose used as a menstrum for lyophilization was shown to protect polysaccharide meningococcal antigens against thermal depolymerization (105, 105, 111). Stabilized meningococcal vaccines in the lyophilized state can be stored at refrigerator temperatures for two years (9, 9a).
Group A polysaccharide vaccine was unaffected by being kept at room temperature (20 - 25°C) for 12 days or at 35°C for 3 days (9a). 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 to 10 weeks at that temperature (9).

A vaccine reconstituted with diluent containing 0.25% phenol was reported to be stable when stored frozen at -20°C for 2 months, or kept at 4°C for 4 weeks, at 25°C for 2 weeks or at 37°C for 4 days (9).

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 (111).

Some stability characteristics of the above vaccines at 37°C are shown in Table 14.

7.2.2 Whole-cell cholera vaccines

Non-adsorbed cholera vaccines inactivated by heat and preserved by thiomersal remain potent in the active mouse protection test at a temperature of 37°C for 1 week (Inaba serogroup component) to 3 weeks (Ogawa component). Neither the Ogawa nor the Inaba component of cholera vaccine adsorbed on aluminium hydroxide showed a significant loss of potency after 4 weeks of storage at 37°C (52).

Longer storage of phenol inactivated and preserved cholera vaccines at elevated temperatures resulted in a clear reduction of potency in mice; after one year storage at 20°C - 25°C and 37°C the mean potency deterioration was around 60% and 92% respectively (44).

There are no published data on the stability of subunit cholera vaccines.

7.2.3 Typhoid vaccine

Whole cell typhoid vaccines inactivated with acetone or heat-phenol seemed to be more thermosensitive than fluid cholera vaccines (52). At 37°C the loss of potency, as tested by the active mouse protection test, was considerable during the first week of exposure but slower thereafter.
Table 14. Stability of non-EPI vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Form</th>
<th>Stability at 37°C</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Estimated potency loss per</td>
<td>Vaccine stable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hour</td>
<td>day</td>
</tr>
<tr>
<td>Meningococcal polysaccharide A + C</td>
<td>L*</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>R**</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Human diploid cell strain rabies</td>
<td>L</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Yeast-derived hepatitis B</td>
<td>R</td>
<td>...</td>
<td>about 2%</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>L</td>
<td>...</td>
<td>3 - 5%</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>50 - 100%</td>
<td>...</td>
</tr>
</tbody>
</table>

* L - lyophilized vaccine
**R - reconstituted or fluid vaccine
8. FINAL CONCLUSIONS

In this paper an effort has been made to assess the loss of potency of various vaccines kept at elevated temperatures. A summary of available information on EPI vaccines is shown in Table 15, where data for various storage temperatures are presented and, in Table 16, where the estimated rates of degradation of EPI vaccines at 37°C are listed.

The stability of EPI vaccines varies considerably. They can be ranked by their resistance to storage at 37°C from stable toxoids to unstable reconstituted measles vaccine or oral polio vaccine (Table 16). 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 to heat, for example, may warrant studies on using this vaccine without refrigeration. Tetanus toxoid could retain sufficient potency for short term exposure to heat when used in the outreach system of delivering immunization against tetanus to women of childbearing age in areas where cold chain cannot be maintained.

However, each exposure to elevated temperature results in some degradation of a vaccine, even if the remaining potency is still above a level which is considered as the minimal immunizing potency. Furthermore, each exposure to ambient temperature has a cumulative impact in reducing vaccine potency: vaccines at peripheral health units will not be able to withstand the temperatures mentioned in this review if their potency has already been compromised by previous breaks in the cold chain. Data presented in this review may provide some guidelines to programme managers and storekeepers at central and provincial levels in making proper decisions about a vaccine exposed to elevated temperatures.

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

The area of greatest need for further research is the heat stability of oral polio vaccine. This is the most sensitive vaccine of the EPI family and it therefore sets the standard for the cold chain. Any improvement in this vaccine would have an influence on the cost and effectiveness of immunization programmes in tropical climates.
### Table 15. Stability of EPI vaccines at various temperatures

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Storage temperature in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 8</td>
</tr>
<tr>
<td>Tetanus and diphtheria toxoids as monovalent vaccines or components of combined vaccines</td>
<td>Stable for 3 - 7 years</td>
</tr>
<tr>
<td>Pertussis vaccine</td>
<td>Safe storage for 18-24 month although with continuous slow decrease of potency</td>
</tr>
<tr>
<td>Freeze-dried BCG vaccine</td>
<td>Stable for one year</td>
</tr>
<tr>
<td>Reconstituted BCG vaccine</td>
<td>Reconstituted BCG vaccine should not be used during more than one working session (5-6 hours). This recommendation has two bases: (1) concern over the risk of contamination, as BCG contains no bacteriostatic agents, and (2) concern over the loss of potency.</td>
</tr>
<tr>
<td>Freeze-dried measles vaccine</td>
<td>Stable for two years</td>
</tr>
<tr>
<td>Reconstituted measles vaccine</td>
<td>Unstable. Should be used in one working session</td>
</tr>
<tr>
<td>Oral poliomyelitis vaccine</td>
<td>Stable for 6-12 months</td>
</tr>
<tr>
<td>Inactivated poliomyelitis vaccine</td>
<td>Stable for 1 - 4 years</td>
</tr>
</tbody>
</table>

Decline of D-antigen content for type I after 20 days
<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Stability at 37°C</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated loss (in%) of potency per</td>
<td>Vaccine stable for</td>
</tr>
<tr>
<td></td>
<td>hour</td>
<td>day</td>
</tr>
<tr>
<td>Tetanus diphtheria toxoids</td>
<td>...</td>
<td>0-1</td>
</tr>
<tr>
<td>Inactivated poliovirus vaccine</td>
<td>...</td>
<td>0.1-3 for type II and III, 0.5-5 for type I</td>
</tr>
<tr>
<td>BCG vaccine Freeze-dried</td>
<td>...</td>
<td>1-6</td>
</tr>
<tr>
<td>Pertussis vaccine</td>
<td>...</td>
<td>1-6</td>
</tr>
<tr>
<td>Measles vaccine freeze-dried</td>
<td>...</td>
<td>2-5</td>
</tr>
<tr>
<td>Reconstituted</td>
<td>10-50%</td>
<td>...</td>
</tr>
<tr>
<td>Oral, live poliomyelitis vaccine</td>
<td>...</td>
<td>20-50</td>
</tr>
</tbody>
</table>
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