WHO Guidance Note: Vaccine Diluents

Revision 2015

THE PROPER HANDLING AND USE OF VACCINE DILUENTS

World Health Organization

This document was published by the Expanded Programme on Immunization of the Department of Immunization, Vaccines and Biologicals

Ordering code: WHO/IVB/15.08

Published in October 2015

This document revises and replaces the WHO Vaccines and Biologicals Update, Volume 34, Proper handling and reconstitution of vaccines avoids programme errors, issued in December 2000 (1).

This publication is available on the Internet at:

http://www.who.int/immunization/documents/en/

Copies of this document as well as additional materials on immunization, vaccines and biologicals may be requested from:

World Health Organization
Department of Immunization, Vaccines and Biologicals
CH-1211 Geneva 27, Switzerland
Fax: + 41 22 791 4227
Email: vaccines@who.int

Design and layout by L'IV Com Sàrl, Villars-sous-Yens, Switzerland.

Printed by the WHO Document Production Services, Geneva, Switzerland.

SUMMARY OF WHO GUIDANCE ON THE PROPER HANDLING AND USE OF VACCINE DILUENTS

A vaccine diluent is the liquid mixed with a lyophilized (freeze-dried) vaccine in order to reconstitute the lyophilized vaccine and provide the final vaccine for administration. A vaccine diluent may be sensitive to heat or freezing, and may require transportation and storage in the cold chain. This guidance note outlines the proper handling and use of vaccine diluent, including, but not limited to, critical steps for reconstituting vaccines safely.

Diluent handling

Diluents vary widely in composition, and therefore only the diluent assigned by the manufacturer for the specific vaccine and presentation should be used.* Never replace a vaccine diluent with water for injection, and never inject an oral vaccine or a diluent used to reconstitute an oral vaccine.

Diluent storage

- Unless otherwise specified by the manufacturer, the correct temperature for long-term storage of diluents is +2°C to +8°C.
  - Diluent packaged with or attached to the vaccine should always be stored with the corresponding vaccine at +2°C to +8°C.
  - Diluent not packaged with the vaccine can be stored at room temperature ONLY if the manufacturer's instructions allow it. In this case, the manufacturer's instructions regarding cooling prior to reconstitution should be followed.
  - Diluents should NEVER be frozen.
- Wherever possible, vaccines and diluents should be stored in a refrigerator that is reserved for this purpose.
- The vaccine vial monitor (VVM) that is attached to the vaccine vial can serve as a visual trigger to assist a health worker in properly applying the multi-dose vial policy, especially in knowing when the reconstituted product must be discarded.
- All diluents should be managed according to standard storage and warehousing practices for vaccines.

Diluent use

- The reconstituted product should be discarded at the end of the immunization session, or within six hours of opening, whichever comes first, UNLESS the reconstituted vaccine meets the criteria for keeping the vaccine for up to 28 days, as indicated in the WHO Policy Statement: Multi-dose Vial Policy (WHO/IVB/14.07).
- Vaccinators should be adequately trained to ensure that the diluent used to reconstitute a vaccine is the correct one assigned by the manufacturer.
  - Appropriate job aids, such as posters, should be provided.
  - Training on the proper handling of diluents should be combined with training on handling multi-dose vials after opening, as specified in the revised WHO Multi-dose Vial Policy. †

* To ensure that a specific vaccine and diluent are approved for use together, refer to the relevant vaccine product sheet on the WHO prequalified vaccines website (http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/index.html, accessed 11 July 2015).

† WHO Policy Statement: Multi-dose Vial Policy (MDVP). Revision 2014. Geneva: World Health Organization; 2014 (WHO/IVB/14.07; http://apps.who.int/iris/handle/10665/135972, accessed 11 July 2015). Individual instructions on handling opened multi-dose vaccine vials can also be found on the WHO Performance, Quality and Safety (PQS) website (http://apps.who.int/immunization_standards/vaccine_quality/pqs_catalogue/, accessed 11 July 2015).

Intended audience

This WHO Guidance Note is designed to provide guidance to national and senior-level programme managers on the proper handling of vaccine diluents and their use in reconstitution. Based on this guidance, aide memoires, training guides and other job aids including supervisory tools should be developed for use at district and facility level to provide more practical guidance on how to safely store, handle and use vaccine diluents.

How to use this document

This Guidance Note explains how to correctly handle and use vaccine diluents. The purpose is to enable vaccinators, logisticians and programme managers to understand how diluents should be used with vaccines and the proper conditions for their appropriate storage and stock management.

This document revises and replaces the WHO Vaccines and Biologicals Update, Volume 34, *Proper handling and reconstitution of vaccines avoids programme errors, issued in December 2000 (1).*

This document is designed to be used in conjunction with *WHO Policy Statement: Multi-dose Vial Policy (MDVP). Revision 2014. Geneva: World Health Organization; 2014 (WHO/IVB/14.07).*

Use of the word diluent in this document

In this document, the term diluent means: a liquid mixed with a lyophilized (freeze-dried) vaccine in order to reconstitute the lyophilized vaccine and provide the final vaccine for administration. In strict pharmaceutical terms, a diluent is an inactive substance such as water for injection *(2)*. In the immunization context, the diluent (liquid used for reconstitution) may also be a liquid containing an adjuvant to enhance immune response or be a vaccine used to reconstitute another vaccine to provide a final combination vaccine. For this reason, a diluent must be strictly used in the recommended quantities, may also be heat or freeze sensitive, and may require transportation and storage in the cold chain *(3)*.

Context

Since 2000, many new vaccines have been included in the Expanded Programme on Immunization (EPI) and many more have been developed. Some of these vaccines are manufactured in lyophilized (freeze-dried) form and must be combined with a diluent before injection. This process is called reconstitution. In certain circumstances, some liquid vaccines may also be available in concentrated formulations and must be diluted with a diluent before administration.

Lyophilization is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapour without passing through a liquid phase. Lyophilization of certain vaccines offers a number of advantages in terms of vaccine production, storage and distribution. This is because vaccines are biological products which are generally heat sensitive, so increased heat stability in a dry state can help to better meet the ambient temperature vaccine transport and storage needs of countries and communities with limited reliable power for refrigeration *(4)*.

Reconstitution must be performed in strict accordance with the manufacturer's instructions. Each diluent is specially formulated for each vaccine, and therefore diluent products are NOT interchangeable. For example, some diluents contain an aluminium adjuvant that is essential to the effectiveness of the vaccine, some contain a preservative, and some are actually a liquid vaccine used to reconstitute a lyophilized vaccine. Examples of different diluents and their composition are given in the Annex.

This Guidance Note takes into account the different requirements of new vaccines introduced since 2000, providing updated guidance on WHO's recommendations for the safe handling of vaccine diluents and their use in reconstitution.

Diluent composition

Because of the variety of diluents available, a vaccinator must be meticulous in verifying that each vaccine is reconstituted ONLY with its assigned diluent in order to ensure that the vaccine is effective. Diluents are formulated specifically for their corresponding vaccine and may contain any or all of the following:

- stabilizers that affect heat sensitivity;
- preservatives to maintain the integrity of the vaccine during storage and distribution;
- bactericides to maintain the sterility of the reconstituted vaccine;
- chemicals to assist in dissolving the vaccine into a liquid;
- buffers to ensure the correct pH balance (level of acidity or alkalinity);
- adjuvants to enhance immune response; and
- a separate and different vaccine.

I. WHO RECOMMENDATIONS ON HANDLING VACCINE DILUENTS

When using vaccine diluents, always bear in mind the following important guidance.

- Never reconstitute a vaccine with a different diluent to the one specified by the manufacturer.
  - Never replace a vaccine diluent with water for injection (see 'Water for injection', below).
  - Never inject an oral vaccine or a diluent used to reconstitute an oral vaccine (see 'Diluents for oral vaccine', below).
- Never freeze a vaccine diluent containing an active substance (see 'Diluents with active substances', below).
- Never use a reconstituted vaccine if foreign particulate matter is observed (see 'Volume of diluents', below).

A. Water for injection

Water for injection should NEVER be used to replace the diluent assigned by the manufacturer. Doing so is a very dangerous practice in terms of both the efficacy and safety of the vaccine, and must be discontinued if practiced.

B. Diluents for oral vaccine

Diluents for oral vaccines tend to be of significantly large volumes per dose, and are not formulated for injection. These diluents may not have sterility characteristics and constituents appropriate for a sterile injection. To avoid serious injury to the recipient, neither oral vaccines nor their assigned diluents should ever be injected.

C. Diluents with active substances

When a vaccine or adjuvanted diluent is used to reconstitute a vaccine, the utmost care must be taken to ensure that the correct diluents are used. Some of these diluents are also freeze sensitive and should therefore be stored and transported in the cold chain with the same care as any other freeze-sensitive vaccine. An example of this is liquid DTP-HepB (do not freeze), which is combined with lyophilized Hib vaccine. To avoid losing the potency of a vaccine, NEVER freeze a diluent containing an active substance.

D. Volume of diluents

Diluents are prepared by the manufacturer specifically for each vaccine and the quantity of diluent is exactly matched to the required volume to arrive at the proper concentration of the product after reconstitution. Therefore, the total contents of the diluent vial must be withdrawn with the reconstitution syringe and added to the vaccine for reconstitution. This may occasionally provide one or two extra doses in the vaccine vial for potential withdrawal excesses, and this is entirely acceptable. These extra doses, if they can be drawn to measure a full dose, may be administered. Note that following reconstitution, the vaccine should be inspected visually for any foreign particulate matter prior to administration. Never use a reconstituted vaccine if foreign particulate matter is observed. In such cases, the vaccine must be discarded and an official report lodged with the supervisor for follow-up investigation.

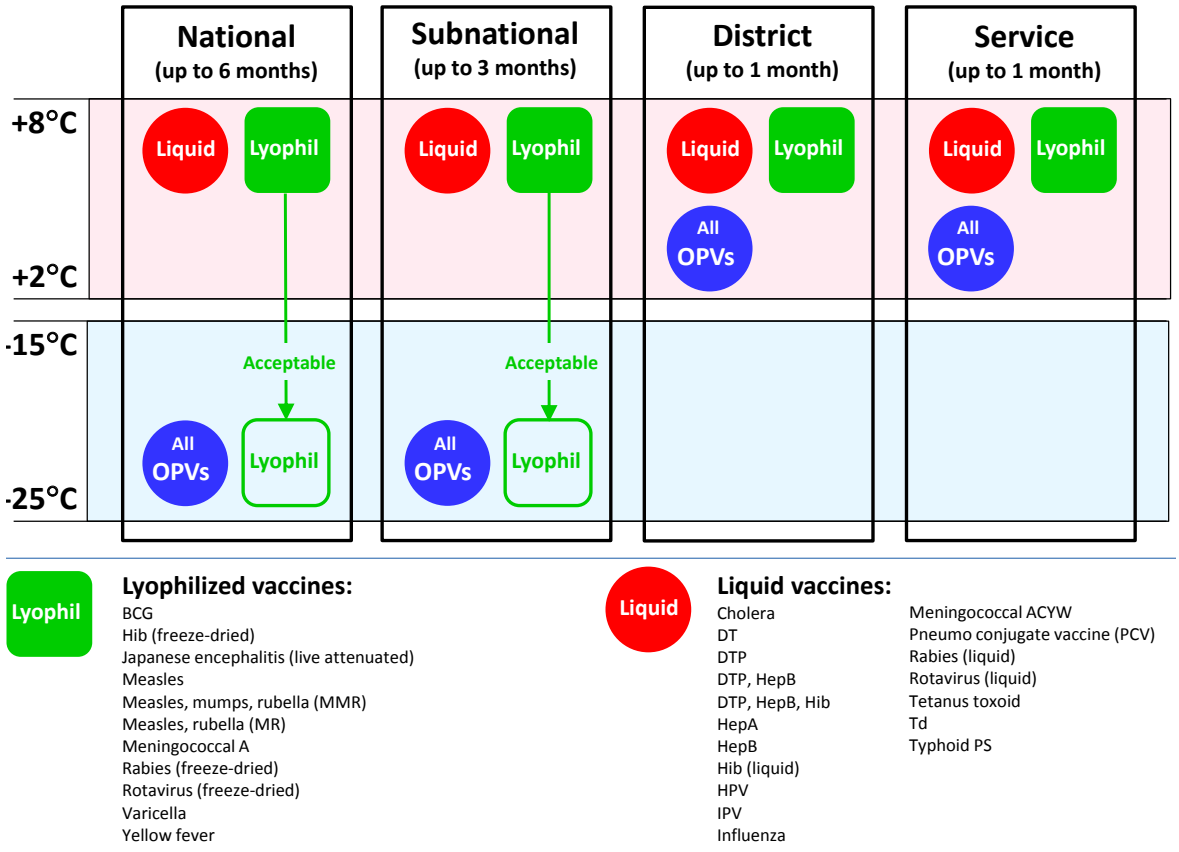
II. PROPERLY STORING AND TRACKING VACCINE DILUENT STOCK

A. Storage of diluents

Unless otherwise specified by the manufacturer, the correct temperature for long-term storage of diluents is +2°C to +8°C. This however is not always practical or cost effective because some diluents can be stored outside the cold chain. In the event that cold chain storage capacity is limited, diluents which are not packaged with the vaccine can be stored at room temperature ONLY if the manufacturer's instructions allow it. In this case, the health worker must take special care to assure that the correct diluent is always used with its designated vaccine, and that the manufacturer's instructions regarding cooling prior to reconstitution are followed. The health worker must also ensure that an adequate number of needles and syringes for reconstitution are available for all the vaccines and diluents.

Diluents should never be frozen. If diluents are packaged with a vaccine, the product should be stored at +2°C to +8°C. Bundled liquid-lyophilized combination vaccines should never be frozen and should also be stored at +2°C to +8°C.

Figure 1. Recommended vaccine storage temperatures and maximum storage periods. *(5)*



1. **Impact of freezing on diluents.** Diluents should never be frozen and should be protected from freezing during transport and storage. There are two main reasons for this. Firstly, the contents of some diluents are sensitive to freezing. For example, diluents that are DTP-containing vaccines are adsorbed onto an aluminium matrix which acts as an adjuvant. Freezing destroys this matrix and thus can lower the efficacy of the product. Such diluents, if suspected of having been frozen, can be tested using the shake test *(6)*. Secondly, the glass vials or glass ampoules in which many diluents are filled are not resistant to freezing and could crack, thereby contaminating the contents and causing unnecessary waste.

2. **Impact of heat on diluents.** If the manufacturer's instructions state that the diluent may be stored outside of the +2°C to +8°C range, the diluent should be cooled to +2°C to +8°C prior to use. The diluent should be cooled to this temperature for at least several hours (and preferably for 24 hours) prior to use for reconstitution. Current formulations of heat sensitive vaccines, but not all, are more stable than in previous years *(5,7,8,9)* and do not require cooling of the diluent. Many vaccines to be reconstituted, especially live attenuated virus vaccines, have only a limited stability when reconstituted, even when kept at +2°C to +8°C *(5,7,10)* and therefore require the diluent to be cooled prior to reconstitution.

Controlled Temperature Chain (CTC)

WHO has defined a Controlled Temperature Chain (11) as a specific set of conditions allowing the transport and storage of a WHO-prequalified vaccine outside the traditional +2°C to +8°C cold chain, for a single excursion at ambient temperatures up to 40°C, for a limited period of time just prior to administration. The vaccine must be licensed and pre-qualified by the appropriate regulatory authorities for use in a CTC, with a label that specifies the conditions. CTC use does not change the use of diluents, except as specified in respective guidance documents, and in accordance with manufacturers' product inserts.

B. Storage of diluents with other medicinal products

Wherever possible, vaccines and diluents should be stored in a refrigerator that is reserved for this purpose *(3)*. If other heat-sensitive supplies, such as drugs, ointments, sera and samples, have to be stored in the same refrigerator, they should be placed in a separate storage container or box in the refrigerator, labelled clearly, and kept completely separate from the vaccines and diluents. Good storage practices indicate that a special area of the refrigerator be reserved for these other products such as the bottom shelf. Note that the fridge should be monitored carefully for acceptable temperature readings according to WHO recommended temperature monitoring principles and appropriate action taken when the acceptable temperature range has been exceeded.

C. Temperature monitoring of diluents

If diluents are stored in the cold chain, the recommendations for temperature monitoring are the same as for vaccines. Effective monitoring and record-keeping achieves the following objectives:

a. verification that storage temperatures are within the acceptable ranges of +2°C to +8°C in cold rooms and vaccine refrigerators;
b. detection of out-of-range storage or transport temperatures so that corrective action can be taken if necessary.

WHO recommends temperature monitoring devices based on the specific cold chain equipment application and the intended monitoring purpose. More detailed information can be found in WHO Vaccine Management Handbook module *How to monitor temperatures in the vaccine supply chain (WHO/IVB/15.04) (12)*.

D. Recording diluents in stock ledgers

When packaged separately from their corresponding vaccine, diluents should be treated as individual products in a stock ledger. Therefore separate stock cards should be prepared for each diluent and should also indicate which vaccine should be reconstituted with it. In the case of computerized stock management programmes, the diluent item should be linked to the appropriate vaccine item for additional stock control and to reduce possible errors in distribution and usage for reconstitution. Each vial received and each vial dispatched/used should be accounted for in exactly the same way as the vaccine and should match the quantity of vaccine vials specific to the diluent. The diluent and its vaccine for reconstitution should always bear the same manufacturer's name although the batch/lot number may differ. It is important to be aware that diluent volumes are normally stated in millilitres on the label for the reconstitution of a vaccine (lyophilized) which is labelled in doses. In the case of freeze or heat sensitivity of the particular diluent (especially when it is another vaccine or adjuvanted diluent), the status of the vaccine vial monitor and the freeze-indicators should also be recorded during receipt and dispatch.

E. Mismatches in quantities of diluent and vaccine

In cases where a mismatch of quantities is found between the diluent and its designated vaccine – this normally occurs during the monthly physical count – the quantities of each should be made equal by one of the following steps described below.

- **If the vaccine stock is short (excess diluent)**
  Step 1. Procure/order more vaccine with the appropriate diluent (this will not decrease the excess of diluent).
  Step 2. Investigate whether the excess diluents can be distributed to another health facility where a shortage of that particular diluent may exist. Remember that diluents are not interchangeable. If this is not possible, then destroy the excess diluents through the locally recognized process for destruction of inventory.
- **If the diluent stock is short (excess vaccine)**
  Step 1. Investigate whether higher-level stores may have excess diluent available. If not, procure/order more diluent from the same vaccine manufacturer.
  Step 2. If the procurement/order of additional diluent only is not possible, then attempt to obtain appropriate diluent from another health facility where excess diluent is available to equalize the quantities. If this not possible, then destroy the excess vaccine through the locally recognized process for destruction of inventory.
  DO NOT USE THE DILUENT OF ANOTHER VACCINE if diluent stock is short.

In any situation where a mismatch of quantities arises, the mismatch should be followed up by careful review of receipt and dispatch vouchers, wastage reports and immunization records to identify the possible reason for the discrepancy. Such a review should also involve the higher- and lower-level stores to determine whether this discrepancy is repeated in other storage sites and thus reflects a system error of procurement, storage or distribution. In some cases it may also reflect a programme error in using the wrong diluent for reconstitution, in which case further investigation will be required.

In cases where a decision has been made to destroy excess vials, remove the excess vials of either the vaccine or the diluent from cold chain storage and clearly label them for destruction. Destroy these excess vials in accordance with the local policy for the destruction of inventory.

III. USING VACCINE DILUENTS SAFELY IN RECONSTITUTION

A. Do not interchange diluents

Vaccinators should be adequately trained to ensure that the diluent used to reconstitute a vaccine is the correct one assigned by the manufacturer. All immunization staff should understand that diluents from different manufacturers are not interchangeable. Below is an illustration of the possible confusion that can occur, based on the varying presentations of lyophilized Hib vaccine. Without accurate stock control, mistakes can easily occur.

VACCINE PRODUCT	VIAL SIZE	RECONSTITUTED WITH	VOLUME OF DILUENT
Product 1	1 dose	0.4% NaCl (saline)	0.5 mL
Product 2	2 dose	0.9% NaCl (saline)	1.0 mL
Product 3	1 dose	DTP-HepB (liquid vaccine)	0.5 mL
Product 4	2 dose	DTP-HepB (liquid vaccine)	1.0 mL

Water for injection should NEVER be used to replace the diluent assigned by the manufacturer.

The following example illustrates the importance of using the correct diluent *(13)*:

Wrongly mixed vaccine can have tragic consequences and undermines trust in immunization services

Country case example
During a measles immunization campaign in 2014, a combination of inadequate training, absence of clear product labelling in the refrigerator and poor supervision led to an error in reconstitution which resulted in the deaths of at least 15 infants. As many as 75 children were injected with a measles vaccine that had been reconstituted with a muscle relaxant (atracurium, intended for use during anaesthesia), which had been stored in the same refrigerator as the vaccine and was mistaken for diluent.

Wherever possible, vaccines and diluents should be stored in a refrigerator that is reserved for this purpose. If other heat-sensitive supplies have to be stored in the refrigerator, they should be placed in a separate storage container or box in the available fridge, labelled clearly, and kept completely separate from the vaccines and diluents.

It is extremely important that when errors in reconstitution occur:
1. The error is investigated with urgency
2. Appropriate action is taken to prevent a re-occurrence
3. Public trust in immunization is promptly restored

B. Open glass ampoules with necessary care and inspection

The process of opening a glass diluent ampoule presents a risk of contamination of vaccine with microscopic glass particles and also possible injury to the vaccinator from the sharp glass. Some of the WHO pre-qualified vaccines/diluents are packed in ampoules.

When opening a glass diluent ampoule, care should be taken to ensure that pieces of glass produced by filing are prevented from getting into the vaccine. Following reconstitution, the vaccine should be inspected visually for any foreign particulate matter prior to administration. If foreign particulate matter is observed, the vaccine must be discarded.

C. Keep reconstituted vaccines cool and discard appropriately

Reconstituted vaccines may become contaminated with unwanted organisms from the outside of the vial and introduced into the inside of the vial during reconstitution or when doses are withdrawn from the vial. These may even be harmful to the recipient if injected. Some vaccines contain a preservative or bactericide to reduce the risk associated with growth of micro-organisms that may have been introduced into the vial, but others do not. For this reason, opened vials of reconstituted vaccines must be kept cool, between +2°C and +8°C, and must be discarded at the end of the immunization session, or within six hours of opening, whichever comes first.

Under certain circumstances, vaccines may be specifically approved by WHO to be kept and used for up to 28 days after opening, according to the Multi-dose Vial Policy. If the vaccine has been approved by WHO for use in a CTC, alternative guidance may apply. Refer to the WHO website for more information on CTC *(11)*.

Reconstituted vaccines to be discarded are classified as infectious non-sharps and should be discarded and destroyed in accordance with the guidelines for the management of solid health-care waste *(14)*.

D. Use Vaccine Vial Monitors as a visual trigger

Vaccine vial monitors (VVMs) are the only temperature monitoring devices that routinely accompany vaccines throughout the entire supply chain *(15,16)*. A VVM is a chemical-indicator label which is applied to a vaccine vial, ampoule or other type of primary container by the vaccine manufacturer. A VVM can be found on nearly all vaccine vials supplied to national immunization programmes procured through UNICEF.

The WHO vaccine prequalification programme has worked with vaccine manufacturers to define VVM placement guidelines so that the VVM, if attached to the vial, can serve as a visual trigger to assist a health worker in properly applying the Multi-dose Vial Policy. This is critically important to knowing when the reconstituted product must be discarded.

There are two different locations for VVMs and each is associated with specific guidance for handling opened **multi-dose vials** of vaccine.

1. *WHO-prequalified vaccines where the **VVM, if attached, is on the label of the vaccine.*** The vaccine vial, once opened, can be kept for subsequent immunization sessions for up to 28 days, regardless of the formulation of the product (liquid or lyophilized).

2. *WHO-prequalified vaccines where the* ***VVM is attached in a different location than on the label (e.g. cap or neck of ampoule).*** In this instance, the vaccine vial, once opened, must be discarded at the end of the immunization session or within six hours of opening, whichever comes first. This is regardless of the formulation of the product (liquid or lyophilized) and would apply, for example, to a reconstituted product of which the vaccine vial cap, which has a VVM attached, has been discarded after opening.

Figure 2. Example of different locations of VVMs on vaccine vials.[1]

E. Check the WHO prequalification website

The range of vaccines prequalified by WHO regularly expands and changes. As a result, WHO routinely updates the individual vaccine product sheets available on the WHO prequalification website *(17)*. To ensure that a specific vaccine and diluent are approved for use together, these individual vaccine product sheets on the website should be consulted.

http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/index.html

This includes the diluent approved for each vaccine (where applicable) and its contents. Follow the View Page link for each vaccine (by manufacturer) where the information and package insert is available.

1 Image courtesy of Temptime. Copyright 2015 Temptime Corporation.

Critical steps for reconstituting vaccines safely

1. Cool the diluent to between +2°C and +8°C, preferably a day prior to its use.
2. Read the label on the diluent to be sure that it is the correct diluent provided by the manufacturer for that specific vaccine and vial size. If the text is written in an unfamiliar language, insist on an accurate local language translation before using.
3. Check the expiry date to make sure that the date has not passed.
4. Check the status of the VVM to make sure that it is not at or beyond the discard point.
5. When opening a glass ampoule, take care to ensure that pieces of glass produced by filing are prevented from getting into the vaccine.
6. Draw all the contents of the diluent into a new sterile reconstitution syringe and empty the entire contents of the diluent into the vaccine vial. Do not try to adjust the quantity of diluent inserted into the vaccine vial because the quantity is precisely calculated to enable removal of the appropriate number of vaccine doses.
7. Draw the fluid slowly and gently in and out of the vial several times to mix the diluent and vaccine or gently swirl the vial; take care not to touch the rubber stopper or opening. Note that following reconstitution, the vaccine should be inspected visually for any foreign particulate matter prior to administration. If foreign particulate matter is observed, the vaccine must be discarded.
8. Do not leave the reconstitution needle in the vaccine vial. Discard the reconstitution syringe in the safety box without recapping.
9. After reconstitution, wrap the vaccine vial in dark paper or foil or insert in a foam pad of a vaccine carrier. Never allow the vial to become wet or immersed in water.
10. Never pre-fill syringes with reconstituted vaccine in order to save time. This is dangerous practice. Vaccines should be prepared individually for each patient.
11. Discard all reconstituted vaccine at the end of the session, or within 6 hours, whichever comes first, UNLESS the product meets WHO criteria for use up to 28 days after opening, in accordance with the Multi-dose Vial Policy (2014).
12. Use a new sterile syringe and needle to withdraw each dose of the vaccine and use this same needle and syringe for injecting the vaccine. After giving the injection, drop the used syringe and needle into the safety box without recapping.

F. Store and distribute diluents in accordance with good practice

All diluents should be managed according to standard storage and warehousing practices for vaccines. This includes regular physical counts of all diluents. Stock ledgers and issue/receipt vouchers for diluents should be in accordance with standard practices for vaccines. These practices for storage, handling and distribution are described in Section II above.

It is important to be aware that diluent volumes are normally stated on the label in millilitres for the reconstitution of a lyophilized vaccine, whose measurement is labelled in doses. Therefore, good record keeping during all stages of storage and transportation, including separate entry for diluents and frequent physical inventory counts, is essential to minimize the risk of using the wrong diluent when matching a vaccine with its diluent.

G. Provide training and job aids

The new practices of using a liquid vaccine to reconstitute another vaccine, the use of adjuvanted diluents, and the reconstitution of oral vaccines, makes it imperative to continue to train health workers and store keepers for good diluent management practices. The proper handling and management of diluents can present significant challenges for the health worker and as a consequence, appropriate job aids, such as posters, should be provided. It is recommended that training on the proper handling of diluents is combined with training on the revised WHO Policy Statement on handling multi-dose vials after opening *(18)*. Such training should be based on the principles articulated in this Guidance Note and should include emphasis on the proper storage temperatures for diluents, critical steps for the safe reconstitution of vaccines, and the placement of VVMs on vaccine vials and their use as visual triggers.

To enable proper supervision of immunization, storage and distribution practices, it is necessary to develop and institutionalize supervisory tools for the handling and management of diluents. Such tools include supervisory guidelines and checklists for use in the field by supervisors and should include as the minimum:

- recording of diluents in stock ledgers;
- recording of temperature monitoring results for diluents;
- actions implemented when temperature excursions were identified;
- identifying mismatches of quantities of vaccines and their diluents;
- reconstitution practices.

Conclusion

The potential impact of these revised recommendations, if rigorously and meticulously applied, is improved safety and efficacy of immunization programmes. When applied in conjunction with the WHO Multi-dose Vial Policy, the impact would include increased savings due to lowered open vaccine vial wastage and improved stock management practices. It is recommended that, in conjunction with the implementation of this revised guidance, WHO and national programmes develop enhanced surveillance for programmatic impact, including monitoring of adverse events following immunization (AEFI) *(19,20)*.

ANNEX

VARIOUS VACCINE TYPES	DILUENT CONTENTS
BCG [BCG(Lyo) + Diluent]	0.9% NaCl (3), Diluted Sauton (1)
Cholera [Cholera(Liq) + Diluent]	Buffer to add to liquid vaccine: sodium carbonate solution with flavouring
DTP-Hib [Hib(Lyo) + Diluent]	DTP vaccine, adjuvanted with aluminium
DTP-Hep B-Hib [Hib(Lyo) + Diluent]	DTP-Hep B vaccine, adjuvanted with aluminium (2)
Hib [Hib(Lyo) + Diluent]	0.4% NaCl (2)
Influenza (pandemic) [Influenza(Lyo) + Diluent]	Adjuvant to add to liquid vaccine (1), Sterile water for nasal inhalation (1)
Measles [Measles(Lyo) + Diluent]	Water for injection (4)
Measles Mumps Rubella [MMR(Lyo) + Diluent]	Water for injection (4)
Measles Rubella [MR(Lyo) + Diluent]	0.9% NaCl (1), Water for injection (1)
Meningitis A conjugate [MenA(Lyo) + Diluent]	Aluminium phosphate (adjuvant) and thiomersal (preservative)
Men AC polysaccharide [MenAC(Lyo) + Diluent]	Buffered saline (2)
Men ACW polysaccharide [MenACW(Lyo) + Diluent]	0.9% NaCl
Rabies [Rabies(Lyo) + Diluent]	Water for Injection (3), 0.4% NaCl (1)
Rubella [Rubella(Lyo) + Diluent]	Water for injection
Yellow fever [YF(Lyo) + Diluent]	0.9% NaCl (3), Water for injection (2)
Rotavirus	Sucrose, disodium adipate, Dulbecco's minimum essential medium
Malaria RTS,S [RTS(Lyo) + Diluent]	S/AS01 (adjuvant)

NOTE: Normal saline is 0.9% Sodium Chloride (NaCl)

REFERENCES

1. Vaccines and Biologicals Update, Volume 34, December 2000. Geneva: Department of Vaccines and Biologicals, World Health Organization; 2000 (www.who.int/immunization/documents/updat34e.pdf, accessed 11 July 2015).

2. Diluent. In: The Free Dictionary [website] (http://medical-dictionary.thefreedictionary.com/diluent, accessed 11 July 2015).

3. Immunization in practice. Module 2: The vaccine cold chain. Geneva: World Health Organization; forthcoming (on publication will be available at http://www.who.int/immunization/documents/training).

4. Lyophilization of Parenteral. Silver Spring (MD): US Food and Drug Administration, Inspections, Compliance, Enforcement, and Criminal Investigations; 2014 (http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074909.htm, accessed 11 July 2015).

5. Temperature sensitivity of vaccines. Geneva: World Health Organization; 2006 (http://whqlibdoc.who.int/hq/2006/WHO_IVB_06.10_eng.pdf, accessed 11 July 2015) (see page 2).

6. Kartoglu U, Ozguler NK, Wolfson LJ, Kurzatkowski W. Validation of the shake test for detecting freeze damage to adsorbed vaccines. Bull World Health Organ. 2010;88:624–31 (http://www.who.int/bulletin/volumes/88/8/08-056879, accessed 11 July 2015).

7. Monath TP. Stability of yellow fever vaccine. Dev Biol Stand. 1996;87:219–25.

8. Chen D, Kristensen D. Opportunities and challenges of developing thermostable vaccines. Expert Review of Vaccines. 2009;8:547–57.

9. Kristensen D. Summary of stability data for licensed vaccines. Seattle (WA): PATH; 2012 (http://www.path.org/publications/files/TS_vaccine_stability_table.pdf, accessed 11 July 2015).

10. Melnick JL. Thermostability of poliovirus and measles vaccines. Dev Biol Stand. 1996;87:155–60.

11. Controlled Temperature Chain (CTC). In: Biologicals [website]. Geneva: World Health Organization; 2015 (http://www.who.int/biologicals/areas/vaccines/controlledtemperaturechain, accessed 11 July 2015).

12. How to monitor temperatures in the vaccine supply chain. Geneva: World Health Organization; 2015 (WHO/IVB/15.04; http://www.who.int/immunization/programmes_systems/supply_chain/evm, accessed 11 August 2015).

13. Statement regarding interim findings of WHO assessments of deaths of children in Idleb Governorate, Syria. Geneva: World Health Organization; 2014 (http://www.who.int/mediacentre/news/statements/2014/interim-findings-idleb-syria, accessed 11 July 2015).

14. Management of solid health-care waste at primary health-care centres: A decision-making guide. Geneva: World Health Organization; 2005 (http://www.who.int/water_sanitation_health/publications/manhcwm.pdf, accessed 11 July 2015).

15. Milstein J. Vaccine vial monitor (VVM) availability and use in the African, Eastern Mediterranean, Southeast Asian, and Western Pacific Regions. Ferney-Voltaire: PATH and World Health Organization; 2010 (http://www.path.org/publications/files/TS_opt_vvm_avail_use.pdf, accessed 11 July 2015).

16. Vaccine vial monitors: Transforming the way we can deliver vaccines [photo essay]. Geneva: World Health Organization; 2015 (http://www.who.int/features/2007/vvm/photo_story, accessed 11 July 2015).

17. WHO prequalified vaccines [website]. Geneva: World Health Organization; 2015 (http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en, accessed 11 July 2015).

18. WHO Policy Statement: Multi-dose Vial Policy (MDVP). Revision 2014. Geneva: World Health Organization; 2014 (WHO/IVB/14.07; http://apps.who.int/iris/handle/10665/135972, accessed 11 July 2015).

19. Adverse events following immunization (AEFI) [website]. Geneva: World Health Organization; 2015 (http://www.who.int/vaccine_safety/initiative/detection/AEFI, accessed 14 July 2015).

20. Vaccine supply and quality: Surveillance of adverse events following immunization. Wkly Epidemiol Rec. 1996;71(32):237–44 (http://www.who.int/docstore/wer/pdf/1996/wer7132.pdf, accessed 11 July 2015).

CONTACT
WORLD HEALTH ORGANIZATION
DEPARTMENT OF IMMUNIZATION, VACCINES AND BIOLOGICALS
20, AVENUE APPIA
CH-1211 GENEVA 27
SWITZERLAND
FAX: + 41 22 791 4227
EMAIL: vaccines@who.int