The cold chain

About this module...

This module describes what the cold chain is, what cold chain equipment is needed in health facilities, and how to use and maintain this equipment.

Some illustrations in this document show equipment from named manufacturers. Their inclusion does not indicate endorsement of a specific manufacturer’s products by WHO.
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Vaccines are sensitive to heat and freezing and must be kept at the correct temperature from the time they are manufactured until they are used. The system used for keeping and distributing vaccines in good condition is called the cold chain. The cold chain consists of a series of storage and transport links, all designed to keep vaccines within an acceptable range until it reaches the user.

Maintenance of the cold chain requires vaccines and diluents to be:

- collected from the manufacturer or an airport as soon as they are available;
- transported between 2°C and 8°C from the airport and from one store to another;
- stored at the correct temperature (see Figure 3A) in primary/central and intermediate vaccine stores and in health facilities;
- transported between 2°C and 8°C to outreach sites and during mobile sessions;
- kept between 2°C and 8°C range during immunization sessions; and
- kept between 2°C and 8°C during return to health facilities from outreach sites.

After vaccines reach the health facility you must:

- Keep them between 2°C and 8°C in your health facility refrigerator.
- Carry them to the immunization session in a vaccine carrier with frozen ice packs or ice.
- Keep the vaccines cool using a foam pad in the vaccine carrier while you immunize the children.

The following figure illustrates the cold chain.
Module 3: The cold chain

Figure 3A: The cold chain

- Vaccine Manufacturer
- International Transport
  - Transit storage facilities (2°C to 8°C)
  - Cold room (2°C to 8°C) and freezer room (-15°C to -25°C)
  - Refrigerators (2°C to 8°C) and freezer room (-15°C to -25°C)
  - Refrigerators (2°C to 8°C) and cold boxes
  - Refrigerators (2°C to 8°C) and cold boxes/vaccine carriers
- National Vaccine Store
- Primary Vaccine Store
- Intermediate Vaccine Store
- Health Centre
- Health Post
- Child and Mother

Transportation with refrigerated trucks and/or cold boxes (vaccine carriers for outreach)
Different levels within the health care system need different equipment for transporting and storing vaccines and diluents at the correct temperature.

- **Primary** vaccine stores need cold or freezer rooms, freezers, refrigerators, cold boxes, and sometimes refrigerator trucks for transportation.
- **Intermediate** vaccine stores, depending on their size/capacity, need cold and freezer rooms, and/or freezers, refrigerators, and cold boxes.
- **Health facilities** need refrigerators with freezing compartments, cold boxes and vaccine carriers.

The cold chain equipment used in health facilities includes the following:

### 2.1 Refrigerators

Health facility refrigerators may be powered by electricity, gas, kerosene, or solar energy. Electric refrigerators are usually the least costly to run and the easiest to maintain, but they must have a reliable electricity supply.

Where the electricity or fuel supply is not reliable, ice-lined refrigerators can maintain the appropriate temperature for 16 hours without power if they operate with power continuously for at least eight hours a day. But the use of ice-lined refrigerators may expose vaccines to the risk of freezing. To prevent an ice-lined refrigerator from freezing vaccines, set the thermostat to 1 and put adhesive tape over the thermostat dial so that it does not get changed, and set the ice-lining switch to “off” (see Figure 3B).

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*Figure 3B: Sticker on ice-lined refrigerator*

This refrigerator contains **freeze-sensitive** vaccines (hepatitis B, DTP, TT, Td, JE, liquid Hib vaccines and their combination).

These vaccines **must not** be frozen!

Set the thermostat to “1”  
Set the ice-lining switch to “OFF”

Put adhesive tape over the thermostat dial

When the power goes off: **DO NOT** adjust the thermostat  
When the power returns: **DO NOT** adjust the thermostat
Bottled gas refrigerators can also keep vaccines at correct temperatures and are easy to maintain. It is difficult to regulate temperatures on kerosene-driven refrigerators and they are also difficult to maintain.

Refrigerators have different capacities for storing vaccines and for freezing and storing ice-packs. A refrigerator in a health facility should be able to hold:

- a one-month supply of vaccines and diluents in the refrigerator compartment;
- a one to two-week reserve stock of vaccines and diluents (an additional 25% to 50% of the one-month supply);
- frozen ice-packs in the freezer compartment; and
- bottles of water or unfrozen ice packs in the refrigerator compartment (to act as a buffer to temperature changes, especially if there is a power failure).

Half the total space in the refrigerator should be left empty to allow air to circulate around the vaccines and diluents to keep them cool.

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**Figure 3C: Two of the most common refrigerators**

- Refrigerator and ice pack freezer, absorption, kerosene/electric
- Compression (electric) refrigerator and ice-pack freezer
2.2 Cold boxes

A cold box is an insulated container that can be lined with ice-packs to keep vaccines and diluents cold during transportation and/or short period storage (from two to seven days).

Cold boxes are used to collect and transport monthly vaccine supplies from district stores to the health facility. They are also used to store vaccines when the refrigerator is out of order or being defrosted and for outreach and mobile sessions in addition to vaccine carriers.

Different models of cold boxes have different vaccine storage capacities. Health facilities usually need one or more cold boxes that can hold:

- a one-month supply of vaccines and diluents; and
- a one–to–two week reserve stock of vaccines and diluents.

In addition to their vaccine storage capacity, cold boxes are selected according to their cold life. Different models have a cold life of two to seven days depending on the temperature outside.

The most suitable cold boxes for a particular health facility are determined by:

- the vaccine storage capacity needed;
- the cold life needed, that is, the longest time that vaccine will be stored in the box;
- the weight and the volume of the box, which depends on how you will transport it – by motor vehicle, bicycle, or hand; and
- ice-packs compatible with size of the cold box.
### 2.3 Vaccine carriers

Like cold boxes, vaccine carriers are insulated containers that, when lined with frozen ice-packs, keep vaccines and diluents cold during transportation and/or temporary storage. They are smaller than cold boxes and are easier to carry if walking. But they do not stay cold as long as a cold box – maximum for 48 hours with the lid closed.

Vaccine carriers are used to transport vaccines and diluents to outreach sites and for temporary storage during health facility immunization sessions. In small health facilities they are used to bring monthly vaccine supplies from the district store. Vaccine carriers are also used to store vaccines when the refrigerator is out of order or is being defrosted.

Different models of vaccine carriers have different storage capacities.

#### The type of vaccine carrier a particular health facility needs depends on:

- the type of vaccines and diluents to be transported;
- the number of vaccines and diluent vials, and ice-packs to be carried;
- the cold life required;
- ice-packs compatible with the size of vaccine carrier;
- the means of transport to be used.

### 2.4 Foam pads

A foam pad is a piece of soft foam that fits on top of the ice-packs in a vaccine carrier. There are some incisions on it to allow vaccines to be inserted in the foam. During immunization sessions, the foam pad serves as a temporary lid to keep unopened vaccines inside the carrier cool while providing a surface to hold, protect and keep cool opened vaccine vials. Previously, ice packs were used to keep vaccines cool during immunization sessions outside of vaccine carriers. It is now recommended to use the supplied foam pads for this purpose.

#### During an immunization session, vials are protected from heat for a longer period of time if they are inserted in a foam pad.
2.5 Ice-packs

Ice-packs are flat, square plastic bottles that are filled with water and frozen. Ice-packs are used to keep vaccines cool inside the vaccine carrier or cold box. The number of ice-packs required for a cold box or vaccine carrier varies. It is recommended to condition ice-packs before using them in a vaccine carrier (see Section 4.2).

Every health facility should have minimum two sets of ice-packs for each of their cold boxes and vaccines carriers:

- one in the process of being frozen
- the other in use in a cold box or vaccine carrier.

Taking ice-packs out of the vaccine carrier will shorten its cold life. During sessions, it is not recommended to keep vaccines on ice-packs or in cups filled with ice to keep vaccines cool. During sessions, stick the vaccine and diluent vials into the foam pad to keep them cool and to protect them.

Ice melts quickly and vials may become contaminated if they float in water from melted ice and labels may fall of the vials. You can avoid this by putting the vials in a sealed plastic bag. Consider open vials that have been under melted water to be contaminated and discard them.
Cold chain monitoring equipment used in health facilities

The purpose of cold chain monitoring equipment is to keep track of the temperature to which vaccines and diluents are exposed during transportation and storage.

3.1 Vaccine vial monitors

A vaccine vial monitor (VVM) is a label that changes colour when the vaccine vial has been exposed to heat over a period of time. Before opening a vial, the status of the VVM must be checked to see whether the vaccine has been damaged by heat.

Manufacturers attach VVMs to vials of most vaccines. The VVM is printed on the vial label or cap. It looks like a square inside a circle. As the vaccine vial is exposed to more heat, the square becomes darker.

Use only vials with inner squares that are lighter in colour than the outside circle. Vials with VVMs in which the inner square has begun to darken but is still lighter than the outer circle should be used before the vials with a lighter inner square.
3.2 Vaccine cold chain monitor card

A vaccine cold chain monitor is a card (different colour background cards exist for different language versions) with an indicator strip that changes colour when vaccines are exposed to temperatures that are too high. The vaccine cold chain card is used to estimate the length of time that vaccine has been exposed to high temperatures.

Manufacturers pack these monitors with vaccines supplied by WHO and UNICEF. Usually the cold chain monitor is only used for large shipments of vaccine. The same card should remain at all times with the same batch of vaccine. The change in color is cumulative and relates to heat exposure over the whole life of the shipment and not to a specific point in the cold chain.

Important note:

VVMs do not measure exposure to freezing temperatures (for freeze-sensitive vaccines).

A VVM not at discard point does not exclude the possibility that the vaccine was frozen. Before use, make sure that the freeze-sensitive vaccine with good VVM has not been frozen.
3.3 Thermometers

Health facility staff use dial or stem thermometers to monitor the temperature of refrigerators.

On a dial thermometer, the needle moves around the scale, pointing to plus (+) numbers when it is warmer and to minus (-) numbers when it is colder.

On a stem or bulb thermometer, coloured fluid in the bulb moves up the scale as it becomes warmer, and down the scale as it becomes colder.

Dial thermometers tend to lose their accuracy over time. Most dial thermometers can be re-calibrated by adjusting a facility screw on the back of the thermometer. To re-calibrate,
match the temperature on the dial thermometer to the temperature shown on a stem thermometer. But to be sure that the dial thermometer still works properly, make a comparison at two different temperatures i.e. inside and outside the refrigerator.

3.4 Freeze indicators

3.4.1 Freeze Watch

A freeze indicator is an irreversible temperature indicator which shows if a product, such as vaccine, has been exposed to freezing temperatures in blue. It consists of a white backing card and a small vial of coloured liquid, all contained in a plastic casing.

If the freeze indicator (Freeze Watch™) is exposed to temperatures below 0°C for more than one hour, the vial bursts and releases the coloured liquid, staining the white backing card.

The freeze indicator is used to warn of freezing and is packed with vaccines that are sensitive to freezing temperatures: DTP, TT, DT, Td (freezing point of -6.5°C), hepatitis B (-0.5°C), liquid Hib and their combinations (DTP-HepB, and DTP-HepB+Hib vaccines) and JE.

Every refrigerator storing vaccines should have a freeze indicator (Freeze Watch™). It is strongly recommended that one freeze indicator be placed in each cold box during vaccine transport and distribution. This is critical in places subject to low temperatures.

Keep the freeze indicator with freeze-sensitive vaccines in the refrigerator. In an upright (front-opening) refrigerator, keep it on the middle shelf, where the freeze-sensitive vaccines and diluents are kept. In a top-opening refrigerator, affix it to the basket in the middle of the refrigerator – not to the side wall, where freezing can occur.

Follow the steps below to read the freeze indicator:

If the indicator paper is stained, your vaccines have been exposed to freezing temperatures.

If the indicator paper shows no colour, remove the indicator from the refrigerator. Shake or tap the edge of the indicator three times on a hard surface. If the paper becomes stained, your vaccines have been exposed to freezing temperatures. If tapping does not cause colour staining in the indicator, put it back into the refrigerator.

1. If the freeze indicator is activated – showing a stain on white background paper – you should perform the shake test on all of the freeze-sensitive vaccines in the refrigerator to determine which ones should be discarded (see Section 8 in this module).
3.4.2 Freeze-tag

Some programmes are using another type of freeze indicator called the Freeze-tag™. It consists of an electronic temperature measuring circuit with associated LCD-display. If the indicator is exposed to a temperature below 0°C ± 0.3°C for more than 60 minutes ± 3 minutes the display will change from the “good” status into the “alarm” status as indicated on the picture below. The indicator is used to warn of freezing and is packed with DTP, TT and DT vaccines as well as with hepatitis B. Shelf life is five years.
How to load cold chain equipment

Cold chain equipment, including refrigerators, cold boxes, and vaccine carriers, must be loaded correctly to maintain the temperature of the vaccines and diluents inside.

4.1 Vaccine refrigerators

Vaccines, diluents, and ice-packs should be kept in a refrigerator that is used only to store them.

If, however, you are in an area with only one refrigerator and you need to store other heat-sensitive supplies such as drugs, ointments, serum, and samples, be sure to label them clearly and keep them separate from vaccines and diluents.

Do not put vaccines on the door shelves. The temperature is too warm to store vaccines, and when the door is opened shelves are instantly exposed to room temperature.

Do not keep expired vaccines, NOR vaccines with VVMs that have reached or are beyond their discard point, NOR reconstituted vaccines for more than six hours or until the end of an immunization session in the refrigerator. Discard them immediately according to your national guidelines. Refer to your supervisor.

Food and drinks should not be stored in a vaccine refrigerator.

Do not open the refrigerator door frequently since this raises the temperature inside the refrigerator.
Vaccine refrigerators have two compartments:

A main compartment (the refrigerator) for storing vaccines and diluents, in which the temperature should be kept between +2°C and +8°C. The thermostat is used to adjust the temperature.

A second compartment (the freezer) for freezing ice-packs. If the refrigerator is working properly, this section will be between -5°C and -15°C.

Load a vaccine refrigerator as follows:

1. Freeze and store ice-packs in the freezer compartment.

2. All the vaccines and diluents have to be stored in the refrigerator compartment. If there is not enough space, diluents can be stored at ambient temperature. It is important, however, that diluents be chilled by putting them in the refrigerator before use.

3. Arrange the boxes of vaccine in stacks so air can move between them; keep boxes of freeze-sensitive vaccine away from the freezing compartment, refrigeration plates, side linings or bottom linings of refrigerators where freezing may occur.

4. If your country has adopted the opened multi-dose vial policy for vaccines, keep opened vials of OPV, DPT, Td, TT, liquid Hib, hepatitis B and DTP-HepB vaccines in the “use first” box for first use during the next session.

5. Keep vials with VVMs showing more heat exposure than others in the box labelled “use first.” Use these vials first in the next session.

6. Only keep vials that are good for use in the refrigerator. Do not include expired vaccines, reconstituted vials with doses remaining after an immunization session, and vials with VVMs that have reached or are beyond their discard point.

7. Keep ice-packs filled with water on the bottom shelf and in the door of the refrigerator. They help to keep the temperature cool in case of a power cut.

Multi-dose vial policy:

Multi-dose vials of DTP, OPV, TT, HepB, DTP-HepB, and liquid Hib from which one or more doses of vaccine have been removed during an immunization session may be used again within four weeks if all of the following conditions are met:

- the expiry date has not passed;
- the vaccines are stored under appropriate cold chain conditions at all times;
- the vaccine vial has not been submerged in water;
- sterile technique has been used to withdraw all doses; and
- the VVM, if attached, has not reached the discard point.
8. Store vaccines in locations appropriate to the style of refrigerator you use. See recommendations below.

**Load front-loading refrigerator with freezer on top (Figure 3M) as follows:**

1. Measles, MR, MMR, BCG and OPV on the top shelf;
2. DTP, DT, Td, TT, HepB, DTP-HepB, Hib, DTP-HepB+Hib, meningococcal, yellow fever, and JE vaccines on the middle shelves; and
3. Diluents next to the vaccine with which they were supplied.
Loading ice-lined refrigerators (ILR) (Figure 3N)

All the vaccines should be stored in the basket provided with the refrigerator

1. Measles, MR, MMR, BCG and OPV in the bottom only; and
2. Freeze-sensitive vaccines (DTP, TT, hepB, DTP-hepB, Hib, DTP-hepB+Hib, meningococcal, yellow fever, and JE vaccines) in the top only.

Figure 3N: Loading top-opening (chest) refrigerators

CAUTION: NEVER ENTER MORE THAN 6 BIG ICE PACKS OR 10 SMALL ICE PACKS PER DAY

4.2 Cold boxes and vaccine carriers

Load vaccines into cold boxes and vaccine carriers as follows:

1. At the beginning of the day of the session, take all the frozen ice-packs you need from the freezer and close the door (see Section 5 of this module for instructions on freezing ice-packs).
Condition frozen ice-packs properly, by allowing ice-packs to sit at room temperature until ice begins to melt and water starts to form. You should check to see if an ice-pack has been conditioned by shaking it and listening for water. This will prevent freeze-sensitive vaccines from freezing.

Put conditioned ice-packs against each of the four sides of the cold box or vaccine carrier and on the bottom of the cold box if required.

Put the vaccines and diluents in the middle of the cold box or carrier.

Include a freeze indicator in the packing with the vaccines.

In vaccine carriers, place a foam pad on top of the conditioned ice-packs. In cold boxes, place conditioned ice-packs on top of the vaccines.

Close the cold box or carrier lid tightly.

Attention:

Although this is not the preferred method, you may need to use ice cubes when you do not have enough ice-packs. If you use ice cubes:

- Put one plastic bag full of cubes in the bottom of the carrier.
- Put the vaccine vials and a freeze indicator in a sealed plastic bag to ensure that labels are not washed away by water from melting ice. Isolate vaccines from the ice with a piece of paper card.
- Do not place ice on top of the vaccines.
- Place a foam pad on top and close the carrier.
How to freeze ice-packs

The proper freezing and use of ice-packs is essential for good quality of the vaccines.

Make sure that the ice-packs you have correspond (sizes and number) to the cold boxes and carriers you are using.

To freeze an ice-pack:

• Fill with water leaving a little air space at the top, and put the cap on tightly.
• Hold each ice-pack upside down and squeeze it to make sure it does not leak.
• Put the ice-packs upright or on their sides in the freezer so that the surface of each ice-pack is touching the evaporator plate, and close the door.
• Gas refrigerators or ice-lined refrigerators with a freezing compartment can freeze up to six large or 12 small ice packs per day. More packs will take longer to freeze.
• Leave ice-packs in the freezer for at least 24 hours to freeze solid.
• After the session put the ice packs back in the freezer.

Keep extra unfrozen ice-packs that do not fit in the freezer on the bottom part of the main refrigerator compartment to keep this section cold in case of a power failure. When you put these ice-packs into the freezer they will freeze relatively quickly because the water inside already is cold. However, do not store already frozen ice-packs in the refrigerator compartment as this will increase the risk of freezing the vaccine.

Remember:

You do not have to refill ice-packs every time you use them. Use the same water repeatedly.

Make sure ice-packs are conditioned (allowed to start melting) before putting them in the cold box containing freeze-sensitive vaccines. This will prevent vaccines from freezing.
How to monitor and adjust the refrigerator temperature

6.1 Monitoring the temperature in vaccine refrigerators

To monitor the temperature of the main section of a refrigerator you need:

- a thermometer; and
- a temperature chart, which you should tape to the outside of the door.

To monitor the temperature, proceed as follows:

- Set the refrigerator thermostat during the coldest part of the day to around +2°C to +4°C.
- Monitor temperatures first thing in the morning and before you leave the post in the afternoon. If the temperature is between +2°C to +8°C, do not adjust the thermostat.
- Continue to monitor the temperature first thing in the morning and before you leave the post in the afternoon, including workdays, weekends, and holidays.
- Record the temperature for the day and time on the refrigerator temperature chart, as shown below.

![Figure 3P: Refrigerator temperature chart](image)
When a chart has been completed, replace it with a new one. Keep the completed charts in a record book for future reference. **Action should be taken when the temperature goes out of range.**

### 6.2 How to adjust the temperature of vaccine refrigerators

If the temperature is too **LOW** (below +2°C):

- Turn the thermostat knob so that the arrow points to a lower number. This will make the refrigerator warmer.
- Check whether the door of the freezer closes properly. The seal may be broken.
- Check freeze-sensitive vaccines (DTP, DT, Td, TT, HepB, DTP-HepB, liquid Hib and DTP-HepB+Hib vaccines) to see whether they have been damaged by freezing by using the shake test (see Section 8 of this module).

**Remember:**

- Slight heat exposure is less damaging than freezing.
- 2°C – 8°C margin is difficult to maintain especially for a kerosene refrigerator.

If the temperature is too **HIGH** (above +8°C):

- Make sure that the refrigerator is working. If not, check if kerosene, gas or power supply is present.
- Check whether the door of the refrigerator or the freezing compartment closes properly. The seal may be broken.
- Check whether frost is preventing cold air in the freezing compartment from entering the refrigerator compartment. Defrost if necessary.
- Turn the thermostat knob so that the arrow points to a higher number. This will make the refrigerator cooler.
- If the temperature cannot be maintained between 2°C and 8°C, store vaccines in another place until the refrigerator is repaired.

**Warning:**

*Do not* adjust thermostat to a higher (cooler) setting after a power cut. This could freeze the vaccines.

*Do not* adjust thermostat to a higher setting when vaccines arrive. This could freeze the vaccines.
If the ice-packs inside the cold box or vaccine carrier have completely melted:

- Discard all reconstituted vials.
- Check VVMs status (see Section 3.1 of this module) and return the vaccines that can be used to a working refrigerator as soon as possible.
- If there is no VVM and the vaccine has only been exposed to warm temperatures for a few hours, return the vials to the refrigerator, place them in the “use first” box, and use them before other vials.
How to maintain cold chain equipment

7.1 Maintaining vaccine refrigerators

A refrigerator works well only if it is properly installed, cleaned and defrosted regularly. Thick ice in the freezer compartment does not keep a refrigerator cool. Instead, it makes the refrigerator work harder and use more power, gas or kerosene. You should defrost the refrigerator when ice becomes more than 0.5 cm thick, or once a month, whichever comes first.

To defrost and clean a refrigerator:

- Take out all the most heat-sensitive vaccines (OPV, measles, BCG, yellow fever) and transfer them to a cold box lined with frozen ice-packs.
- Take out all the freeze-sensitive vaccines (DTP, DT, Td, TT, hepatitis B, liquid Hib, DTP-HepB) and diluents, and transfer them to a cold box lined with conditioned ice-packs.
- Turn off the power supply to the refrigerator.
- Leave the door open and wait for the ice to melt. Do not try to remove the ice with a knife or ice pick, since doing so can permanently damage the refrigerator. You can place a pan of boiling water inside and close the door.
- Clean the inside of the refrigerator and door seal with a clean wet cloth.
- Turn the refrigerator on again.
- When the temperature in the main section falls to +8°C or lower (but not less than +2°C), return the vaccines, diluents, and ice-packs to their appropriate places.

If you need to defrost your refrigerator more than once a month, it could be because:

- you may be opening it too often (more than three times daily); or
- the door may not be closing properly; or
- the door seal may need to be replaced.
7.2 What to do when a vaccine refrigerator is out of order

If your vaccine refrigerator stops working, first protect the vaccines and then repair the refrigerator.

Protecting the vaccines

Move the vaccines to another place until the refrigerator is repaired. If you think that the problem will last only a short time, you may use a cold box or vaccine carrier lined with conditioned ice-packs for temporary storage. For a longer duration, use another refrigerator. Always keep a freezer indicator with the freeze-sensitive vaccines to monitor eventual freezing.

Restoring the refrigerator to working order

Check the power, gas or kerosene supply. If there is no power, make other arrangements (e.g. store the vaccine in a household refrigerator) until power is restored. If there is no gas or kerosene, get it as soon as possible.

If a lack of power, gas or kerosene is not the problem, repair the refrigerator or report to your repair technician or supervisor.

Record the breakdown on the daily temperature recording chart.

Note: Concerning the routine maintenance and the servicing of refrigerators, WHO technical manuals exist for each kind of refrigerator.

7.3 Maintaining cold boxes and vaccine carriers

Vaccine carriers and cold boxes must be well dried after their use. If they are left wet with their lids closed, they will become mouldy. Mould may affect the seal of the cold boxes and vaccine carriers. If possible, store cold boxes and vaccine carriers with the lid open, when not being used.

Knocks and sunlight can cause cracks in the walls and lids of cold boxes and vaccine carriers. If this happens the vaccines inside will be exposed to heat.

If a cold box or vaccine carrier wall has a small crack you may be able to repair it with adhesive tape until you can get an undamaged one.
The shake test

The “shake test” can help give an idea whether adsorbed vaccines (DTP, DT, Td, TT or hepatitis B) have been subjected to freezing temperatures likely to have damaged them. After freezing, the vaccine no longer has the appearance of an homogenous cloudy liquid, but tends to form flakes which settle at the bottom of the vial after shaking. **Sedimentation is faster in a vial which has been frozen than in a vial, from the same manufacturer, which has not been frozen.**

The test should be conducted for all boxes where freeze indicators are found to be activated or temperature recordings show negative temperatures.

**Procedure:**

1. **Prepare a frozen control sample**

   Take a vial of vaccine of the same type and batch number as the vaccine you want to test, and from the same manufacturer. Freeze the vial until the contents are solid (at least 10 hours at -10°C) and then let it thaw. This vial is the control sample. Mark the vial clearly so that it is easily identifiable and will not be used by mistake.

2. **Choose a test sample**

   Take a vial (s) of vaccine from the batch (es) that you suspect has been frozen. This is the test sample.

3. **Shake the control and test samples**

   Hold the control sample and the test sample together in one hand and shake vigorously for 10–15 seconds.

4. **Allow to rest**

   Leave both vials to rest by placing the vials on a table and not moving them further.

5. **Compare the vials**

   View both vials against the light to compare the sedimentation rate. If the test sample shows a much slower sedimentation rate than the control sample, the test sample has most probably not been frozen and can be used. If the sedimentation rate is similar, the vial has probably been damaged by freezing and should not be used.

   Note that some vials have large labels which conceal the vial contents. This makes it difficult to see the sedimentation process. In such cases, turn the control and test vials upside down and observe sedimentation taking place in the neck of the vial.

   If the shake test procedure indicates that the test sample has been damaged by freezing, you should notify your supervisor immediately. Identify and separate all vaccines that may have been frozen and ensure that none are distributed or used.
Note:

Frozen samples can be used for shake tests only when testing the same vaccine from the same manufacturer and the same lot number. A new sample is needed for each manufacturer and lot number.

Figure 3R: The shake test

Compare the deliberately frozen vial next to the suspect vial

<table>
<thead>
<tr>
<th>Deliberately frozen vial</th>
<th>Suspect vials</th>
</tr>
</thead>
<tbody>
<tr>
<td>almost clear</td>
<td>USE THIS VACCINE</td>
</tr>
<tr>
<td>thick sediment</td>
<td>DO NOT USE THIS VACCINE</td>
</tr>
</tbody>
</table>

If the sediments in the suspect vial settle more slowly, the suspect vaccine may be used.

If the sediments in the suspect vial settle at the same rate, the suspect vaccine may NOT be used.
Summary

The tables below show the sensitivity of different vaccines to heat and freezing:

**Table 3.1: Heat sensitivity**

<table>
<thead>
<tr>
<th>Range</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>most sensitive</strong></td>
<td>OPV</td>
</tr>
<tr>
<td></td>
<td>Measles, MR, MMR</td>
</tr>
<tr>
<td></td>
<td>DTP, DTP-HepB, DTP-Hib, DTP-HepB+Hib, YF</td>
</tr>
<tr>
<td></td>
<td>BCG</td>
</tr>
<tr>
<td></td>
<td>Hib, DT</td>
</tr>
<tr>
<td><strong>least sensitive</strong></td>
<td>Td, TT, HepB, JE</td>
</tr>
</tbody>
</table>

**Table 3.2: Freeze sensitivity**

<table>
<thead>
<tr>
<th>Range</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>most sensitive</strong></td>
<td>HepB</td>
</tr>
<tr>
<td></td>
<td>Hib (liquid)</td>
</tr>
<tr>
<td></td>
<td>DTP, DTP-HepB, DTP-Hib, DTP-HepB+Hib, YF</td>
</tr>
<tr>
<td></td>
<td>DT</td>
</tr>
<tr>
<td></td>
<td>Td</td>
</tr>
<tr>
<td><strong>least sensitive</strong></td>
<td>TT, Hib lyophilised</td>
</tr>
</tbody>
</table>

**Light sensitivity**

Finally, some vaccines are very sensitive to strong light and their exposure to ultraviolet light causes loss of potency. Consequently, they must always be protected against sunlight or fluorescent (neon) light. BCG, measles, MR, MMR and rubella vaccines are equally sensitive to light (as well as to heat). Normally, these vaccines are supplied in vials made from dark brown glass, which gives them some protection against light damage, but care must still be taken to keep them covered and protected from strong light at all times.