

Standard Operating Procedures

Packaging and transportation of *Bacillus anthracis* culture

Project

“DBT Network Programme on Anthrax Diagnosis and Control in India”



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Anthrax is a bacterial disease caused by the spore-forming bacterium *Bacillus anthracis*, a Gram-positive, rod-shaped bacterium and the only obligate pathogen in the large genus *Bacillus*. *Bacillus anthracis* (*B. anthracis*) is a zoonotic organism that can result in four types of infection, depending on the pathogen's point of entry into the body via inhalation, cutaneous, gastrointestinal, and injection. *B. anthracis* (anthrax) can contaminate soil, plants, and water. Animals can be infected when they breathe or ingest spores.

Anthrax is an infectious material that was previously used in bioterrorism, so we required more attention to the packaging and transportation of *Bacillus anthracis* culture.

Packaging and transportation of culture

The packaging material used for Category 'A' substances requires more stringent specifications (**UN2814**), such as pressure tested at 95 kPa, drop tested from 9 meters, puncture tested at 7 kg and stacking tested.

Triple packaging system

Each completed package is normally required to be correctly **marked, labeled** and accompanied with appropriate shipping **documents** (as applicable).

Packing requirements

Packing Requirement	Substance
Inner primary containers	
Leak proof primary (1 ^o) and secondary (2 ^o) containers	yes
Pressure-resistant 1 ^o or 2 ^o container	yes
Positively sealed 1 ^o container	yes
Absorbent between 1 ^o and 2 ^o containers	yes
List of contents between 2 ^o and outer package	yes
Rigid outer packaging	yes
Name and number of responsible person (must be placed on the Outer package)	yes
Markings and labels with emergency number	yes

Quantity Limits for Either Passenger or Cargo Aircraft	
Maximum for each 1 ^o container	50 mL/50 g
Maximum total for each outer package	50 mL/50 g
Documentation	
Shipper's Declaration for Dangerous Goods	yes
Emergency response telephone number	yes

Inner primary containers and materials

- ✓ A primary container must be a watertight, leak proof (for liquid material) or sift proof (for solid material) receptacle.
- ✓ Primary containers may be glass, metal or plastic but must be leak proof and sealed.
- ✓ Liquids transported by air should have an appropriate air space inside the receptacle.
- ✓ Absorbent material (e.g., cotton wool) sufficient to absorb all liquid or solid hazardous contents contained within the primary container(s) in case of breakage must be placed between the primary and secondary containers.

Inner secondary containers and materials

- ✓ A leak or sift proof secondary container that contains the primary container(s) and absorbent material, such as a zip-lock style specimen transport bag containing a biohazard symbol.
- ✓ Secondary containers can be rigid or flexible, must be of adequate size to hold the primary receptacle, and contain absorbent material and cushioning (required if multiple or fragile primary receptacles).
- ✓ An itemized list of the contents and quantities of the primary container(s) must be attached to or included on the outside of the secondary container and not within.

Outer/tertiary containers and materials

- ✓ Secondary packaging is placed in outer shipping packaging with suitable cushioning material.

- ✓ It should have a resistant, high-density external cover (e.g., metal, wood, or fireboard), shock absorbent padding on the inside, and a tight-fitting lid.

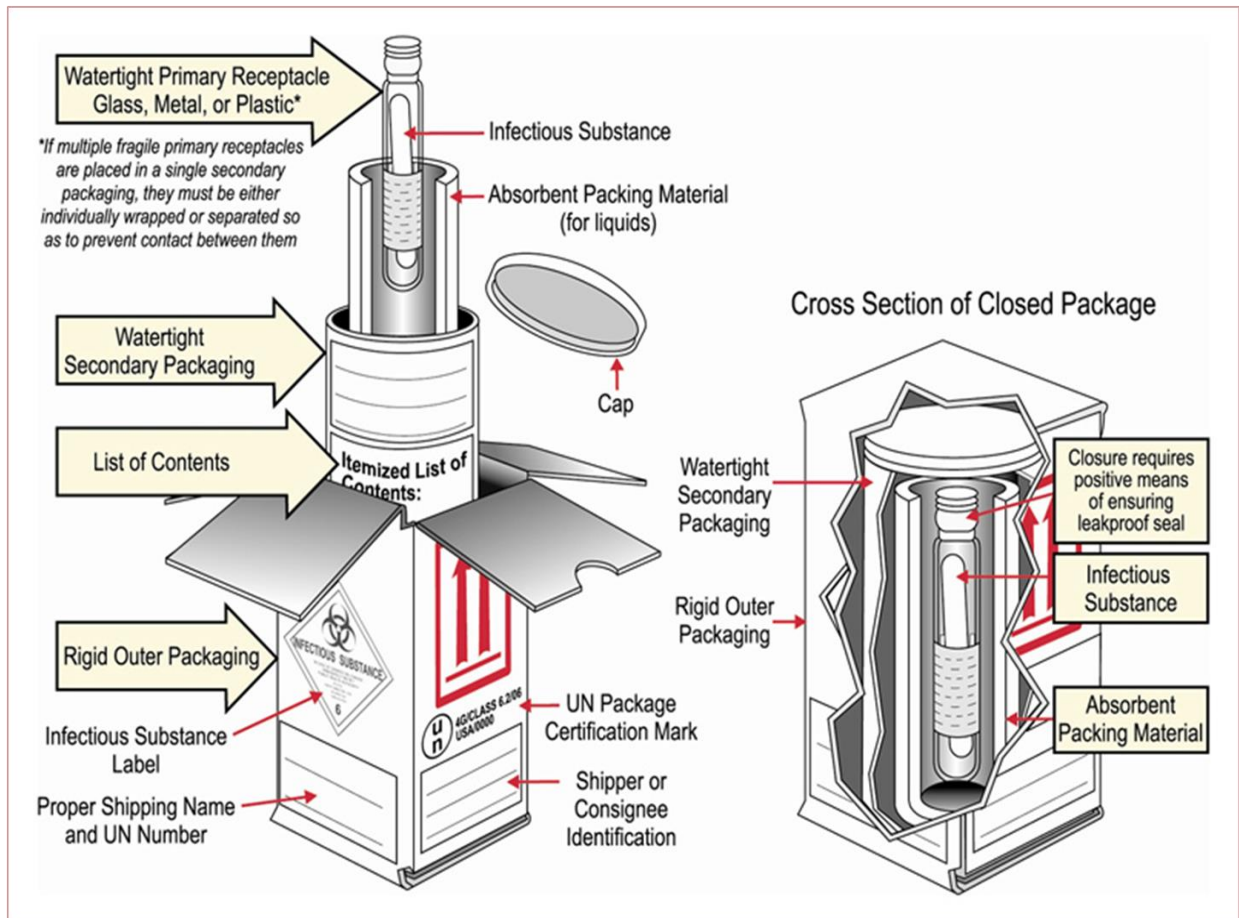


Fig.:- Triple packaging system

- ✓ The outer package must be leak proof and well insulated and can contain ice, cold packs or dry ice when needed.
- ✓ Vaccine carriers or other commercially made containers may be used as tertiary containers for transport.
- ✓ The outer surface must be of adequate size to place all required markings (Biohazard marking compulsory) and labels on one side.
- ✓ Orientation labels (up arrows) must be placed on opposite sides of all packages that contain >50 mL of a liquid or frozen liquid infectious substance to indicate the correct orientation of the package.

- ✓ For surface transport, there is no maximum quantity per package. For air transport, the limits per package are as follows: 50 ml or 50 g for passenger aircraft and 4 liters or 4 kg for cargo aircraft.

Marking of packages

All markings on packages shall be placed in such a way that they are clearly visible and not covered by any other label or marking.

Each package shall display the following information on the outer packaging.

- ✓ The shipper's (sender's, consignor's) name and address
- ✓ The telephone number of a responsible person knowledgeable about the shipment
- ✓ The receiver's (consignee's) name and address
- ✓ Proper shipping name ("INFECTIOUS SUBSTANCE, AFFECTING HUMANS AND ANIMALS" or "BIOLOGICAL SUBSTANCE CATEGORY A", etc., as appropriate).
- ✓ Technical names need not be shown on the package.
- ✓ Temperature storage requirements (optional)
- ✓ When dry ice or liquid nitrogen is used: the technical name of the refrigerant and the net quantity.
- ✓ Orientation labels ("UP" arrows) shall be affixed to two opposite sides of the outer packaging.

Labeling

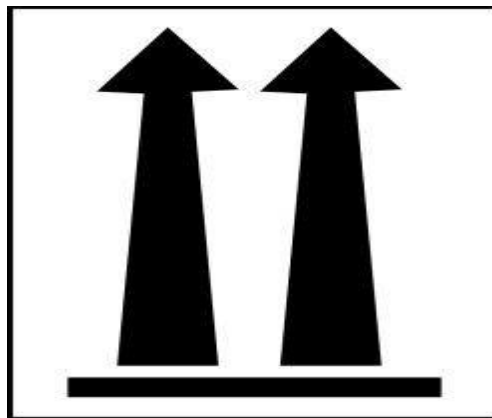
- ✓ Hazard labels should be placed in the form of a square set at an angle of 45° (diamond-shaped)
- ✓ Minimum dimensions: 100 × 100 mm (for small packages: 50 × 50 mm)
- ✓ No. of labels per package: 1
- ✓ Color: Black and white

- ✓ The words “INFECTIOUS SUBSTANCE” with the statement “In case of damage or leakage immediately notify a Public Health Authority” should be shown.



Orientation label

- ✓ Minimum dimensions: 74 × 105 mm
- ✓ No. per package: 2 on opposite sides
- ✓ Color: Black and white or red and white
- ✓ The words “THIS SIDE UP” or “THIS END UP” may also be displayed on the top cover of the package.



Precautions

- ✓ Before transport, notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival.
- ✓ Securely fasten transport boxes in the transport vehicle.

- ✓ Keep spill kits in the vehicle containing absorbent materials, chlorine disinfectant, heavy duty reusable gloves, mask, and apron and leak proof waste disposal containers.
- ✓ Keep extra refrigerant in the vehicle (minimum of 4 ice packs will maintain refrigeration for 2-3 days) in case of delay in the travel schedule so that the cold chain is maintained.
- ✓ Preferably, they should be transported in cool boxes.
- ✓ If using dry ice, the outer packaging used must be breathable to release the CO₂ that will build up in the package.

Required Material

Package: Primary, Secondary, outer/ tertiary

Shock absorbent: Cotton

Leak prevention: Parafilm tape, packaging tap

Accessories: Scissors, Marker, Tag, Biohazard and other symbols

Documentations: Sender and receiver full details, Emergency number

Process of packaging

1. Prepare culture on the nutrient slant in the 15 ml disposable sterile screw-capped tube and incubate overnight.
2. All up to secondary packages required materials take place in the BLS-3.
3. First, we will start with securing the culture tube; checking any leakage/breakage, cleaning the outside tube area with spirit, sealing with parafilm tape, and marking the culture ID.
4. The culture tube will be placed in the primary container and cap side surely to the upper side.
5. Cotton/absorbent filled between the primary container and culture tube then closed container and sealed with parafilm tube.

6. After that, the primary container shifts into the secondary container and is fixed with absorbents.
7. Then close the secondary container and sealed with parafilm.
8. Now, we can transport secondary container outside from the BSL-3 through pass box.
9. Secondary container shifts into outer package and documents are placed between them.
10. Finally, closed the outer package with tape.
11. All marking and labeling are done properly for smooth and secure transportation.

Receiving Process

1. Information from the sender, do all necessary arrangements to receive and process the culture.
2. Culture to be received by the Principal Investigator/Co-PI or SRF under this project (By name).
3. Culture will be placed under the lock in Refrigerator.
4. Inform the sender after receiving samples/send a copy to the sender RECEIPT IN RECEIPT REGISTER.
5. OPENING: On arrival, all lab work is performed in a BSL-3 laboratory using a Class II Biosafety cabinet.
6. NOTE Check the leakage or any other damage to the outer (tertiary) packaging container.
7. NOTE Check the leakage or any other damage to the secondary packaging container.
8. Remove the documents from the container and make the required entries in the receiving register and provide the receiving register number to all the culture isolates.
9. Open the secondary container in Class II BSC using BSL-3 instructions.
10. Primary container is now carefully opened, and quality of the container and gross exam checked.
11. Complete all the entries of the preliminary quality register.

12. Discard all the rough materials, including secondary packaging taking all bio-safety measures.
13. Store the culture tubes in the sample cabinet of the refrigerator and process further for bacteriology study.
14. Complete the logbook of the refrigerator, and after locking, keys submitted to P.I.