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Royal Government of Bhutan  
Ministry of Agriculture and Forests  
Department of Livestock  
NATIONAL DAIRY RESEARCH CENTRE  
Yusipang, Thimphu



## GUIDELINES ON HANDLING LIQUID NITROGEN & FROZEN BOVINE SEMEN (CODE OF PRACTICE)

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*Prepared by :*

*Dr.Lham Tshering, Dr.Dorji, Mr.Mohan Gurung & Mr.Phub Dorji*

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## **Layout and Design**

Lokey Thapa  
Yuka Nakanishi  
NDRC, Yusipang

## **Contact Address**

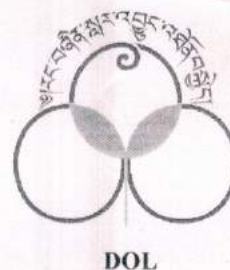
Specialist Head/ Program Director  
National Dairy Research Centre (NDRC)  
Department of Livestock, MoAF  
Yusipang, Thimphu, Bhutan

PO Box: 1058  
Phone: Office: +975 17116985, 17116976(PABX) Lab: 17116986  
Email address: [ndrc@moaf.gov.bt](mailto:ndrc@moaf.gov.bt)

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ROYAL GOVERNMENT OF BHUTAN  
Ministry of Agriculture and Forests  
Department of Livestock  
Thimphu



### FOREWORD

I am pleased to note that National Dairy Research Centre (NDRC), Yusipang has come-up with guidelines on handling of Frozen Bovine Semen and Liquid Nitrogen (LN<sub>2</sub>). This is one of the cryogenic liquids used in preserving frozen semen at -196°C used for Artificial Insemination Program in the country.

This guidelines are to inform handlers/users on ways to prevent hazards associated with LN<sub>2</sub> handling that include “*extreme cold*” whereby the vapour of liquid nitrogen can rapidly freeze skin tissue and eye fluid, resulting in cold burns, frostbite, and permanent eye damage even by brief exposure.

Handlers/users are to be aware that LN<sub>2</sub> is liquefied under high pressure and can expand to a very large volume of gas. LN<sub>2</sub>, the colourless, odourless, tasteless, and deadly gas when it vaporizes has no warning properties. Hence, if sufficient LN<sub>2</sub> is vaporized it reduces oxygen percentage putting person handling it at a risk of oxygen deficiency. Further, equipment containing these cryogenic fluids must be kept clear of combustible materials in order to minimize the fire hazard potential.

These guidelines provide tips for Safe handling of LN<sub>2</sub> and its containers during transportation and storage; provide guide to determine volume of LN<sub>2</sub> level in the semen bank and proper handling of frozen semen and maintenance of cold chain. I am sure these guidelines will be useful for handlers/users to take necessary precaution to prevent risk associated while handling LN<sub>2</sub> and costly frozen semen.

(Dr. Tashi Samdup)  
**DIRECTOR GENERAL**

Director General  
Department of Livestock  
Ministry of Agriculture & Forests  
Thimphu : Bhutan

POST BOX NO.113  
POSTAL CODE: 11001

PABX:- 1975-2-322418/322795/FAX: 335400; Director-323146; PA to Director-322384, FAX: 322094; Livestock Production Division:324933 ;  
Livestock Health Division:322443; Animal Nutrition Division: 322443; Bio-Gas: 331410/332040 FAX: 331418; GOI Project: 335168/FAX:  
335167

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# **GUIDELINES ON HANDLING OF LIQUID NITROGEN (LN<sub>2</sub>) AND FROZEN BOVINE SEMEN**

## **(CODE OF PRACTICE)**

### **Summary**

This guidance is intended to provide information on Liquid Nitrogen (LN<sub>2</sub>) and Frozen Bovine Semen handling and code of practice in the field. The contents of this guidance should be familiar for all users of LN<sub>2</sub> and Frozen Bovine semen. This guideline should be supplemented by appropriate training and demonstration where specific tasks are undertaken.

### **Properties and safety precautions while handling Liquid Nitrogen**

- ⊙ LN<sub>2</sub> is a cryogenic liquid used in cryopreservation of bovine semen for Artificial Insemination (AI). LN<sub>2</sub> is a non-toxic, non-inflammable, invisible colourless, odourless liquid with a boiling point of -196 °C. At low temperatures the gas / vapour is heavier than air. Small amounts of liquid vaporize rapidly to produce large volumes of gas (1 litre of LN<sub>2</sub> will produce 0.7 m<sup>3</sup> of gas). Nitrogen gas is invisible; the cloudy vapour which appears when LN<sub>2</sub> is exposed to air is condensed moisture, not the gas itself.
- ⊙ LN<sub>2</sub> is very cold (-196 °C) extremely hazardous to handle and may cause asphyxiation and cryogenic burns (frost bite). Nitrogen vapour if inhaled can cause hypoxia leading to respiratory distress. Continuous evaporation of LN<sub>2</sub> in poorly ventilated room causes decrease in atmospheric Oxygen which may result in asphyxia / suffocation.
- ⊙ Ensure adequate ventilation of room when working with LN<sub>2</sub>. Avoid all skin contact with LN<sub>2</sub> as direct exposure of body surface / skin to LN<sub>2</sub> may lead to cryogenic burn.
- ⊙ Use only properly specified equipment like LN<sub>2</sub> Containers for storing and transporting LN<sub>2</sub>.
- ⊙ Wear protective clothing (Glasses & gloves) while handling LN<sub>2</sub>.
- ⊙ Do not introduce warm objects in LN<sub>2</sub>, this may lead to boiling, splashing and gasification.
- ⊙ LN<sub>2</sub> should not come in contact with rubber / plastic or materials which cannot withstand temperature of -196 °C.
- ⊙ Wash with plenty of warm Water area of body that is accidentally splashed with LN<sub>2</sub>. If skin burn occurs, flood affected area with cold H<sub>2</sub>O & apply cold compress. Seek immediate medical attention if eyes have been affected or the skin is blistered. If dizziness or loss of consciousness occurs due to lack of oxygen, move the person to well ventilated area.

# GUIDELINES FOR HANDLING OF LIQUID NITROGEN & FROZEN SEMEN

## *I. SAFETY HANDLING OF LN<sub>2</sub> CONTAINERS DURING STORAGE AND TRANSPORT*

- ⊙ Liquid Nitrogen (LN<sub>2</sub>) containers are metal (Aluminium or Stainless steel) double walled (Inner & Outer) vacuum vessels with efficient insulation system. The Vacuum space is completely free from air molecules. The Inner chamber, which contains LN<sub>2</sub> is suspended from outer shell by neck tube (joint/welding between inner & outer vessel at point of neck).
- ⊙ LN<sub>2</sub> Containers should be stored below 50°C (for increasing the holding time, *i.e* total days required for complete evaporation of LN<sub>2</sub>)
- ⊙ It is always advisable to store LN<sub>2</sub> containers in a cooler place.
- ⊙ Avoid direct exposure of the containers to sun rays / hot air blast.
- ⊙ LN<sub>2</sub> Containers should always be stored in an upright position. Tipping the container or letting it lie on its side will result in spillage and may damage the container or the materials stored in it.
- ⊙ Keep LN<sub>2</sub> Container in Clean Dry place on wooden plank or jute carpet to avoid direct contact with hard surfaces. Contact with wet / damp surface / concrete / cement floor and chemicals will cause corrosion & ultimately lead to vacuum loss.
- ⊙ Keep Container vertically (upright position) in well ventilated room as poorly ventilated room may lead to suffocation due to decrease in atmospheric Oxygen. Do not store in a confined space.
- ⊙ Always keep Container closed with neck plug/cap and do not store LN<sub>2</sub> for long periods in an uncovered container to minimize its loss.
- ⊙ While opening, slide out neck plug vertically to prevent possible damage of neck plug cap. Use specific neck plug/caps & canisters recommended for the individual model of Container. Improper fitting of the neck plug/caps and Canisters may stick to neck tube due to frost formation.
- ⊙ Fill dry container and handle LN<sub>2</sub> slowly and carefully to minimize boiling and splashing. Boiling and splashing always occur when charging or filling a warm container with cryogenic

liquid or when inserting objects into it. Handle the liquid using specified forceps to withdraw frozen semen immersed in cryogenic liquid.

- ⦿ Avoid all sorts of shocks like dropping /dragging / rough handling. Extensive shock may create a pendulum type vibration in the inner vessel which may lead to breakage at neck tube junction resulting in partial or complete vacuum loss. Don't tilt / roll Container to avoid splashing of LN<sub>2</sub>. This will also prevent dents & scratches which lead to vacuum loss.
- ⦿ Lift Containers with both hands & set it down gently. For containers that cannot be easily and safely carried, a trolley can provide safe and easy movement of containers.
- ⦿ Do not stack LN<sub>2</sub> Containers one above other as it may result in inadequate release of gaseous Nitrogen causing pressure built up in Container which may lead to explosion.
- ⦿ Avoid welding, brazing, drilling or punching on container which will cause permanent damage to container by way of vacuum loss.
- ⦿ The primary hazards of transferring liquid nitrogen from one container to another are spilling and splashing. Use of big size plastic funnels will reduce splashing and wastage. Follow carefully the instructions on containers or accessories when transferring LN<sub>2</sub>. Never overfill the containers. Filling above the specified level is likely to produce spillage when the neck tube core is replaced.
- ⦿ Never seal LN<sub>2</sub> in a container, store it in containers with loose fitting lids. A tightly sealed container will build up pressure as the liquid boils and may explode after a short time.
- ⦿ Check LN<sub>2</sub> containers regularly for evidence of frost on outside the container and excessive loss of LN<sub>2</sub> which indicates a breakdown of insulation.
- ⦿ Closed trucks or vans are not recommended for transporting LN<sub>2</sub> containers as adequate ventilation is required to prevent nitrogen gas from accumulating.
- ⦿ LN<sub>2</sub> containers should be secured in an upright position and protected from heavy jolting or colliding with one another during transport to prevent spillage, tipping over & damage to the containers.



## II. DETERMINE VOLUME OF LN<sub>2</sub> LEVEL IN THE SEMEN BANK

- ⦿ Extremely low temperature of the LN<sub>2</sub> or nitrogen gas provides the protection of the materials stored in cryogenic storage containers. When all of the LN<sub>2</sub> has evaporated, the temperature inside the container will rise slowly. The rate of evaporation depends upon the age, condition and use pattern of the container. Opening and closing the container or moving it about will reduce its cooling efficiency.
- ⦿ Check the LN<sub>2</sub> level in the semen bank at least weekly; make sure there is enough LN<sub>2</sub> in the container to maintain the required temperature to avoid damage to the semen straws stored in the container.
- ⦿ If the liquid has evaporated faster than usual or if the container is covered with frost or condensation, the vacuum system may be damaged. In such instances, transfer the contents to another container and remove the damaged one from service at once.
- ⦿ The LN<sub>2</sub> level in the semen bank can be determined by Dip stick method
- ⦿ For this, slowly insert Dip stick up to bottom of container vertically, Keep it for 5 to 10 seconds, take out the Dipstick and wave it in air. There will be frost formation. Measure length of frosted section, which indicates level of LN<sub>2</sub> remaining in the container.



**Always maintain minimum of 10  
cm of LN<sub>2</sub> level in semen banks**



### III. PROPER HANDLING OF FROZEN SEMEN AND MAINTENANCE OF COLD CHAIN

- ⊙ Proper semen handling is essential to maintain optimum reproductive performance of AI program in the field. Frozen semen can be stored indefinitely, if it is maintained constantly at very low temperatures. Semen must be kept at temperatures well below critical temperatures (-80 °C). Exposure to increased temperatures can occur when semen is transferred from tank to tank or when handling semen within the neck while trying to locate semen straws. Thermal injury to sperm is permanent and cannot be corrected by returning semen to LN<sub>2</sub>. The strict semen handling practices are recommended to minimize thermal damage.
- ⊙ Transfer of semen between tanks must be coordinated and rapid. Two people should be involved, and tanks should be arranged side by side. If possible, fill the tanks with LN<sub>2</sub> before transfer.
- ⊙ When removing semen straw from LN<sub>2</sub> tank, it is imperative that canister, cane, & unused semen straws are kept as low as possible in the neck of semen bank. Raise canisters only to a level necessary to locate the appropriate semen in the goblet.
- ⊙ It is best to keep semen from one bull in each goblet. Such systems help avoid unnecessary searching and exposure of semen to dangerously high temperatures within the neck region.
- ⊙ Prepare to transfer semen by raising the canister into the lower portion of the neck where the desired semen in the goblet can be grasped. Lower the canister further into the neck. Secure the goblet as low as possible in the neck to protect the other straws from thermal damage. If the straw cannot be easily removed from the plastic goblet, lower and immerse the goblet in LN<sub>2</sub> and repeat the process again.
- ⊙ Keep all unused straws below frost line in neck of tank.
- ⊙ Temperature in the neck is important as sperm injury (as judged by sperm motility) occurs at temperature as low as -79°C. Furthermore, injury to sperm cannot be corrected by returning semen to LN<sub>2</sub>. In semen tank, dangerous temperatures exist in the upper half of the neck tube. Exposure to these temperatures can occur when trying to locate a specific unit of semen or when transferring semen from tank to tank.

- ◎ To maintain cold chain and keep semen well below critical temperature, it is imperative to refill semen banks in the AI centres every 45 to 50 days interval.
- ◎ The transport LN<sub>2</sub> containers with net capacity (51.5 litres), used for transporting LN<sub>2</sub> to the AI Centres should not be exchange with the Reserve LN<sub>2</sub> container with net capacity (34 litres) at the AI Centres. The transport containers with higher capacity should be used only for schedule distribution of LN<sub>2</sub>.





**NATIONAL DAIRY RESEARCH CENTRE**  
**DEPARTMENT OF LIVESTOCK**

**MINISTRY OF AGRICULTURE AND FORESTS**

**YUSIPANG, THIMPHU, BHUTAN, POST BOX : 1058**

**☎17116985 (Direct), 17116986 (Lab), 17116976 (PABX) / [www.ndrc.gov.bt](http://www.ndrc.gov.bt)**