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Review

INTERVENTIONAL STUDY OF LIQUID NITROGEN ON BREAST CANCER BY CRYOABLATION TECHNIQUE

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Abstract

Breast cancer remains a major cause of cancer-related morbidity and mortality worldwide, creating a need for minimally invasive and cosmetically acceptable treatments. Cryoablation, a percutaneous technique using extreme cold to destroy malignant tissue, has emerged as an alternative to surgery in selected early-stage cases. This study examines the use of liquid nitrogen (−196°C) in breast cancer cryoablation, focusing on its mechanisms, procedure, material compatibility, dosing, and therapeutic outcomes. Freezing induces ice crystal formation, cellular dehydration, vascular damage, and tumor necrosis, while potentially stimulating anti-tumor immunity. Clinical evidence, including ICE3 trial results, reports a 96.39% five-year local recurrence-free survival rate in low-risk tumors under 1.5 cm, with minimal complications and excellent cosmetic outcomes, supporting its safety and effectiveness in carefully selected patients.

Keywords: Breast Cancer, liquid Nitrogen, Cryoablation

INTRODUCTION OF CANCER

Cancer is among the deadliest diseases affecting the both people and animals. It occurs due to the uncontrolled, rapid, and irregular proliferation of cells, statistics show that cancer is second most common cause of death worldwide, after cardiovascular diseases, with around 20million people diagnosed and about 9.7 million deaths (48.5%) linked to cancer in 2015.Cancer is the name given to cancer, which can be either cause rapid or delayed cell proliferation.

BREAST CANCER

- Breast cancer is the most commonly diagnosed cancer among women globally, with an estimated 297,790 new cases of invasive carcinoma and 55,720 new cases of ductal carcinoma in situ (DCIS) in 2023.
- As surgical interventions for breast cancer become less aggressive, non-surgical options are being investigated for early-stage cases with favorable characteristics (such as low grade and hormone receptor positivity). These options include various imaging-guided, minimally invasive techniques like cryoablation, radiofrequency ablation, microwave ablation, high-intensity focused ultrasound, laser therapy, vacuum excision, and irreversible electroporation.

Types of breast cancer:

- Invasive
- Non invasive

LIQUID NITROGEN:

Liquid nitrogen (In) is best experienced in an unazoted state. Its boiling point is around -196˚C (-321˚F). It is generated industrially through the fractional distillation of liquid air. This colorless and fluid liquid has a viscosity roughly one-tenth that of acetone (for 30 different liquids at room temperature), and it is commonly utilized as a coolant.

PURITY LEVEL OF LIQUID NITROGEN:

How is nitrogen gas purity classified?

The purity of nitrogen is evaluated through various methods, such as chromatography or analytical techniques using gas analyzers that quantify purity in parts per million. This assessment can occur before the nitrogen is introduced into a pipeline system or designated for ultra-high purity (UHP) nitrogen. The production of UHP nitrogen involves advanced gas processing techniques, including pressure swing adsorption (PSA) and membrane separation.

Ultra-High Purity (UHP) Nitrogen Gas

UHP Nitrogen typically consists of 99.999 to 99.9999% nitrogen, with contaminants usually found in parts per billion (ppb). This grade is commonly used in industries like semiconductor and pharmaceutical manufacturing.

Liquid Nitrogen (LN_2): A High Purity Cryogenic Fluid for Various Applications high-purity Liquid Nitrogen (LN_2) suited for industrial, scientific, medical, and laboratory applications requiring controlled ultra-low temperatures and certified safety standards. Produced through advanced air separation and cryogenic distillation processes, Liquid Nitrogen provides exceptional purity (up to 99.999%), stable cooling performance, and conforms to international standards.

High purity liquid nitrogen cryogenic products go through testing and certification per ISO 14175, EN 13648, ISO 17025, and NIST reference standards, ensuring reliability and traceability for every delivery.

Industries Using Ultra-High Purity (UHP) Nitrogen:

UHP nitrogen enhances performance, improves safety, and reduces contamination risks in cryogenic applications critical in sectors where even tiny impurities can compromise the final product.

Key industries include:

Semiconductor Manufacturing: Provides a controlled environment essential for fabrication processes.

Pharmaceuticals: Helps prevent contamination and oxidation during drug production.

Food and Beverages: Food-grade UHP nitrogen displaces oxygen in packaging to inhibit oxidation and prolong shelf life of products like snacks and wine.

APPLICATION:

- Laboratories and Research: Supports sample preservation, spectroscopy, and material testing under cryogenic conditions.
- Medical and Biological Use: Essential for applications in cryotherapy, dermatology,

and cryopreservation of biological samples, tissues, and cells.
- Pharmaceutical and Biotech: Maintains cold-chain integrity for vaccines, reagents, and genetic materials.
- Food and Beverage Industry: Applied for rapid freezing, cooling, and controlled atmosphere packaging.
- Engineering and Manufacturing: Utilized in superconductivity studies, cryogenic pipe testing, and liquid cooling systems.
- Liquid Nitrogen in dewars, cryogenic tanks, and bulk storage systems, ensuring long-term stability and operational safety

Advantages of High purity Liquid Nitrogen
- High Purity (up to 99.999%) for safe use across medical, research, and food sectors.
- Ultra-Low Temperature (-196°C) ideal for cryogenic applications.
- Certified Quality, with every batch analyzed in an ISO 17025-accredited lab and traceable to NIST standards.
- Safe Handling and Delivery options, including portable dewars, stationary tanks, or bulk systems.
- Certificate of Analysis (CoA) accompanying each batch, verifying purity and compliance with ISO 14175 / EN 13648.

LOW PURITY NITROGEN GAS:

Low purity nitrogen gas can be divided into several categories:
- Compressed Air: A mix of gases including nitrogen, oxygen, and trace amounts of other gases, often unsuitable for applications requiring pure nitrogen.
- Commercial Grade Nitrogen (CGN): Typically contains 95% to 99.999% nitrogen, with impurities like oxygen and carbon dioxide.
- High Purity Nitrogen (HPN): Usually consists of 99.999% to 99.9999% nitrogen, with impurities at parts per million (ppm) levels, often used in electronics manufacturing and laboratory testing.

EVALUATION TEST FOR LIQUID NITROGEN:

Liquid Nitrogen Testing Procedure

PURPOSE

To develop a standardized method for assessing the purity and quality of liquid nitrogen (LN_2) through the evaluation of:
- Physical properties
- Oxygen contamination
- Moisture and particulate contamination

SCOPE

This procedure is relevant for LN_2 used in:
- Cryopreservation
- Analytical instruments
- Microbiological and pharmaceutical fields
- Food and semiconductor laboratories

PRINCIPLE

Pure liquid nitrogen has a boiling point of −196 °C, is colorless, odorless, and contains at least 99.999% nitrogen. Any contaminants, such as oxygen, moisture, hydrocarbons, or particulates, can affect its boiling behavior, color, density, or oxygen levels.

MATERIALS & EQUIPMENT
- Cryogenic Dewar flask
- Cryogenic thermocouple (−200 °C range)
- Oxygen analyzer (in ppm)
- Analytical balance
- Clean stainless-steel ladle
- Approved cryogenic container
- Moisture indicator / dew point analyzer (if available)

SAMPLE COLLECTION

Collect LN_2 directly from the supply Dewar using a cryogenic ladle and immediately transfer it to a clean, labeled Dewar for testing.

TEST PARAMETERS & PROCEDURES

Visual Inspection

Procedure:

Examine the appearance of LN_2 in good lighting.

Acceptance Criteria:

Must be clear, colorless, and free from turbidity or suspended particles, and should not have a bluish tint (which indicates oxygen contamination).

Boiling Point / Temperature Test

Procedure:

Insert a cryogenic thermocouple into the LN_2 and record the stable temperature.

Acceptance Criteria:

Temperature should be between −195 °C and −197 °C.

Density / Weight Check (Optional)

Procedure:

Measure a known volume of LN_2 and determine its mass using an insulated container.

Acceptance Criteria:

Density should be approximately 0.807 g/mL at −196 °C.

Oxygen Contamination Test

Procedure:

Let LN_2 evaporate in a closed sampling hood and measure the oxygen concentration using an analyzer.

Acceptance Criteria:

Oxygen levels should be ≤ 10 ppm for research grade and ≤ 2 ppm for IVF/semiconductor grade.

Moisture (Water Vapor) Test

Procedure:

Analyze the evaporated gas using a dew-point analyzer or moisture sensor.

Acceptance Criteria:

Moisture levels should be ≤ 5 ppm.

Particulate Matter Test

Procedure:

Allow LN_2 to evaporate in a clean vessel and visually inspect the vessel surface.

Acceptance Criteria:

No visible residues or particulates should be present.

RESULT RECORDING

PHYSICAL & CHEMICAL TEST RESULTS

S.NO	Parameter	Unit	Result	Acceptance criteria	Remarks
1.	Appearance	--	Clear,Colorless	Clear, Colorless	PASS
2.	Boiling temperature	C °	-196	-195 to -197	PASS
3.	Density	g/ml	0.803	0.807	PASS
4.	Oxygen Content	ppm	1.85	≤10ppm	PASS
5.	Moisture Content	ppm	3	≤ 5ppm	PASS
6.	Particular Matter	--	--	Nil visible	PASS

Microbial Examination of Liquid Nitrogen

PURPOSE

To outline a standardized method for the microbiological examination of liquid nitrogen (LN_2) via indirect testing, aimed at detecting microbial contamination that may occur during handling, storage, or transfer.

SCOPE

This Standard Operating Procedure (SOP) is applicable to LN_2 intended for:

- Cryopreservation (IVF, cell lines, stem cells)
- Pharmaceutical and GMP laboratories
- Food and microbiology labs
- Research and diagnostic applications

PRINCIPLE

Liquid nitrogen itself does not support microbial growth due to its extremely low temperature (−196 °C). Any microbial contamination, if present, typically comes from:

- Testing condensate after LN_2 has evaporated
- contact surfaces

PERSONAL PROTECTIVE EQUIPMENT (PPE)

- Cryogenic gloves
- Face shield or safety goggles
- Lab coat
- Closed shoes
- Sterile gloves for microbiological tasks

MATERIALS & EQUIPMENT

- Sterile cryogenic Dewar or container
- Sterile stainless-steel ladle
- Sterile swabs
- Sterile collection vessels
- Plate Count Agar (PCA)
- Sabouraud Dextrose Agar (SDA)
- MacConkey Agar (if needed)
- Tryptic Soy Broth (TSB)
- Fluid Thioglycollate Medium (FTM)
- Incubators (25 °C, 30–35 °C, 37 °C)
- Laminar air flow cabinet
- Autoclave

SAMPLE COLLECTION

Condensate Collection Method:

Transfer LN_2 into a sterile container and allow it to evaporate completely at room temperature. Collect the condensed liquid aseptically for immediate microbial analysis.

Swab Method (Dewar & Accessories):

Moisten a sterile swab with sterile saline and swab the inner surfaces of the Dewar neck, lids, and ladles. Place the swab into sterile saline or broth.

TEST PROCEDURES

Total Viable Count (TVC)

Method: Pour plate / spread plate

Medium: Plate Count Agar

Procedure:

Inoculate 1 mL of condensate onto PCA, incubate at 30–35 °C for 48–72 hours, then observe and count colonies.

Acceptance Criteria:

Not Detected / ≤ 1 CFU/mL

RESULT RECORDING

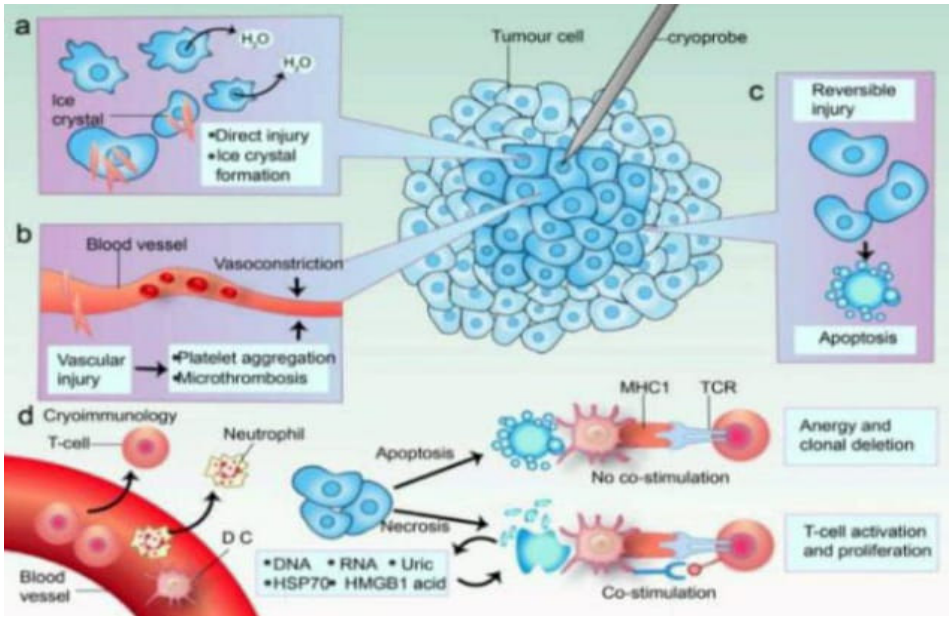
Test Parameter	Result	Acceptance Criteria	Pass/Fail
Total Viable Count	No growth	≤ 1 CFU/mL	PASS

CRYOABLATION TECHNIQUE:

Cryoablation is a minimally invasive procedure that employs extreme cold to freeze and destroy tissues via the insertion of a cryoprobe. This probe is typically cooled using substances such as liquid nitrogen, liquid nitrous oxide, or compressed argon gas.

MECHANISM:

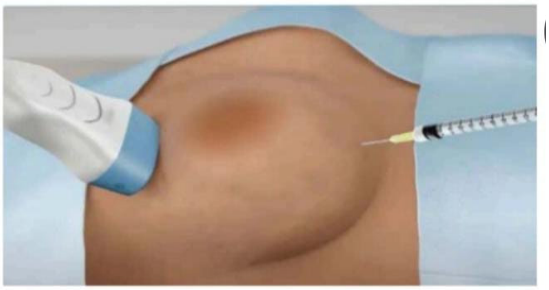
The effectiveness of cryoablation in eliminating tumors is linked to several vital processes. The extreme cold causes ice crystals to form within the tumor cells, damaging their membranes and organelles, leading to cell death through necrosis. Rapid cooling can also trigger an inflammatory response that enhances the immune system's ability to recognize and attack tumor cells. Additionally, the necrotic tissues release specific tumor antigens into the bloodstream, potentially stimulating both innate and adaptive immune responses. The immunological aspect is particularly crucial when combining cryoablation with immunotherapy, as it may increase overall effectiveness against tumors while minimizing damage to surrounding healthy tissue through real-time imaging guidance, such as ultrasound or MRI.



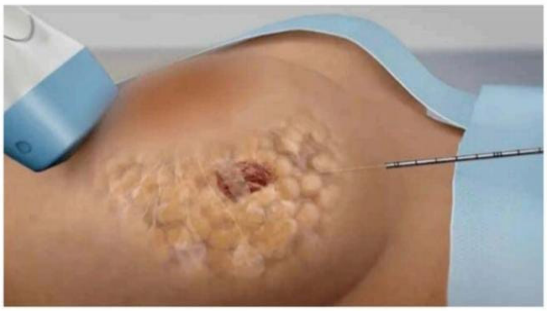
Mechanisms of cell death in cryoablation:
Cryoablation causes a central zone of frozen necrosis with immediate tissue destruction. In this region, temperatures fall below −40°.Extreme cold leads to rapid extracellular ice crystal formation.Ice formation creates a hypertonic extracellular environment.cells lose water and undergo osmotic shrinkag.Intracellular ice crystals further damage cellular structures. Cold exposure also injures blood vessels and endothelial cells.Endothelial disruption promotes platelet aggregation and microthrombi formation.Vasoconstriction and increased vascular permeability contribute to ischemia.The resulting lack of blood flow produces coagulative necrosis.In surrounding areas exposed to milder cold, reversible injury may trigger apoptosis.Immune cells infiltrate the tissue through affected blood vessels.Apoptotic tumor cells can be cleared silently without strong T cell activation.These dying cells may release immunosuppressive cytokines such as IL-10 and TGF-β, promoting T cell anergy or deletion.Alternatively, necrotic tumor cells release intracellular contents that stimulate inflammatory and immune responses

Cryoablation Procedure
During the procedure, guided by CT or ultrasound imaging, a cryoprobe is inserted into the tumor. A coolant (cryogen) is then delivered to the active area of the probe within a closed-loop system, which cools the probe and generates an ice ball around the affected tissue. As the ice ball forms, intra- and extracellular ice crystals develop, leading to cell death. A repetitive freeze-thaw-freeze cycle promotes further ice crystal formation, weakening cell membranes and causing their rupture. This process also inhibits blood flow from nearby capillaries, depriving any surviving cells of nutrients and preventing regrowth. The body's immune system subsequently clears away the necrotic tissue.

Step 1: Local Anesthesia
To ensure comfort during the procedure, a local anesthetic is injected into the skin and inner breast area where the cryoprobe will be inserted. While the injection may cause brief discomfort, it is quickly followed by numbness at the site.



Step 2: Cryoprobe Insertion
A tiny incision (around 3 mm) is created in the skin. The cryoprobe, which resembles a needle, is inserted through this opening and directed to the tumor’s center with the help of ultrasound. Accurate placement of the cryoprobe is essential for successful treatment



Step3: Freezing the tumor

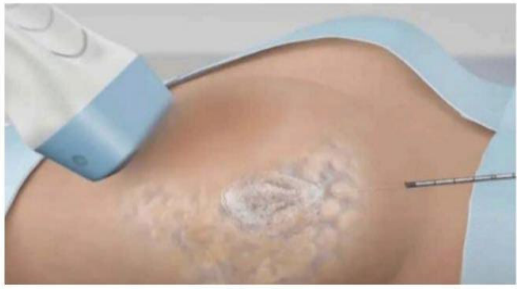
Liquid nitrogen flows through the cryoprobe, producing extreme cold at its tip.

This process freezes the tumor along with an area of normal tissue, reaching a core temperature of -180˚C at the tumor's center and -40˚C at its surface.

The freezing procedure consists of three cycles: 10 minutes of freezing, although the durations for freezing and thawing can change depending on the circumstances.

Step 4: Completion of Cryoablation

Following the second freezing cycle, the cryoprobe is heated and removed from the breast. To reduce bleeding, pressure is applied to the skin opening, and a small bandage is applied over the incision.



APPLICATION OF CRYOABLATION:

Cancer Treatment:

Cryoablation is extensively employed for various cancer, particularly when surgery is not feasible due to tumor size location or patient conditions.

Pain and Symptom Management:

It helps alleviate pain other symptoms for cancer that have metastasized, particularly to bone or other organs.

Cardiac Arrhythmias:

Cryoablation is utilized to treat irregularly heart rhythms by freezing problematic cardiac tissue to restore normal electrical conduction.

CONCLUSION:

Liquid nitrogen- based cryoablation offers a safe, effective, and minimally invasive therapeutic option for selected early-stage breast cancers, particularly small, low-risk, hormone receptor-positive tumors. Its ability to achieve complete tumor destruction through rapid freeze- thaw cycle, while preserving surrounding healthy tissues, provides oncologic control comparable to breast- conserving surgery in specific patient groups.

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