Ultra-Freeze Shipment Validation Summary

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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1 Introduction

This document justifies the addition of an insert cardboard tray and a secondary flat cardboard box inside the DI-4 box to ensure and maintain the positioning of the frozen product in the core of the box (well surrounded by dry ice). **The end objective is to ensure that temperature is maintained over shipment of Ultra-Freeze products.**

1.1 Objectives

1.1.1 Thermal performance of the ultra-freeze shipment packaging

This report summarizes the shipment performance of the DI-4 box packaging solution, combined with an insert cardboard tray and a secondary flat cardboard box inside.

The performance of the packaging concept is characterized among others by the measurement of the temperature inside the flat cardboard box where the shipped product is placed.

The aim of this study is to provide evidence that packaging configurations are capable of:

- maintaining the product well positioned inside dry ice
- maintaining required temperature during the shipment of the product

1.1.2 Functional resistance of the packaging (ASTM D4169)

This report summarizes the conditions and tests done to verify the functional resistance of the DI-4 packaging for product shipped in Styrofoam box filled with dry ice, in a cardboard box (called DI-4 box).

Tests were performed by an accredited subcontractor, according to ASTM D4169 DC13 severity level 2 standard, which represents worst case shipping corresponding to air freight + road transportation. They consist in:

- Drop tests
- Dynamic compression
- Low frequency vibrations
- Depression
- Random vibrations
- Drop tests

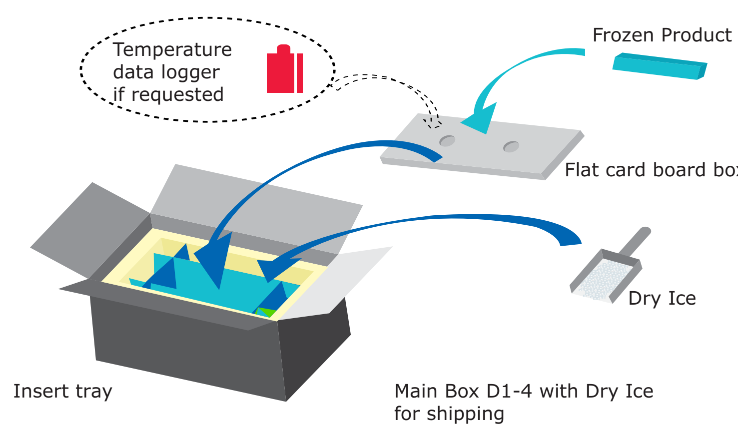
The aim of this study is to provide evidence that the packaging protects the final product from any damage that would prevent the product use.

1.2 Presentation of the object

1.2.1 Product packaging for transportation and shipping

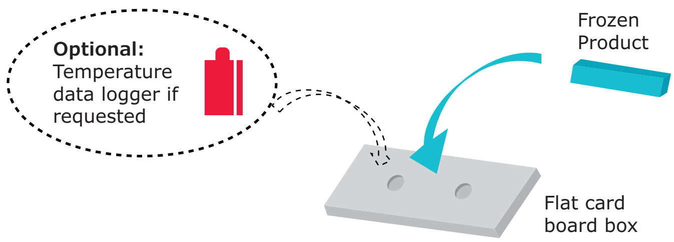
1.2.1.1 General information on the packaging process

The transportation of frozen products needs specific requirements and must be performed into dry ice. The standard process is the use of DI-4 box to have enough dry ice for a transit time of maximum 5 days. To guarantee required temperature of dry ice during transportation, the packaging needs to ensure the products are well positioned into the core of the box and surrounded by dry ice. The concept of the optimized packaging for shipment is the following:

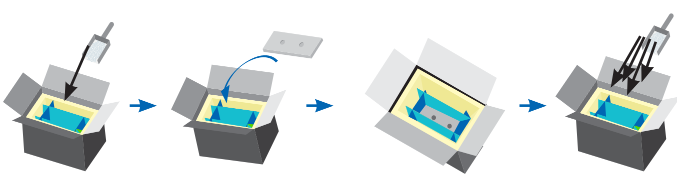


Optimization brought to the packaging:

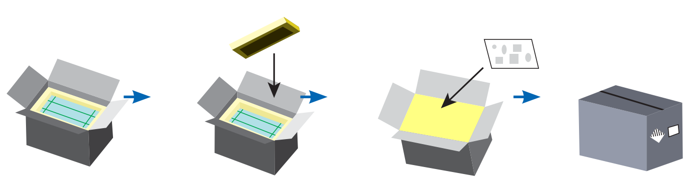
As secondary packaging was added: the frozen product is now placed into a flat cardboard box.



1. An insert cardboard tray was added in the DI-4 box (the DI-4 box, considered as the third packagng, is composed of polystyrene and carboard)
2. Then, a small quantity of dry ice is added in the DI-4 box. The secondary flat cardboard box is placed in the middle of the insert trays. It is placed directly onto dry ice and is then covered and surrounded by dry ice. This insert is intended to maintain the secondary flat cardboard box containing the frozen product in the middle of the DI-4 box to ensure that it remains well covered with dry ice during shipment

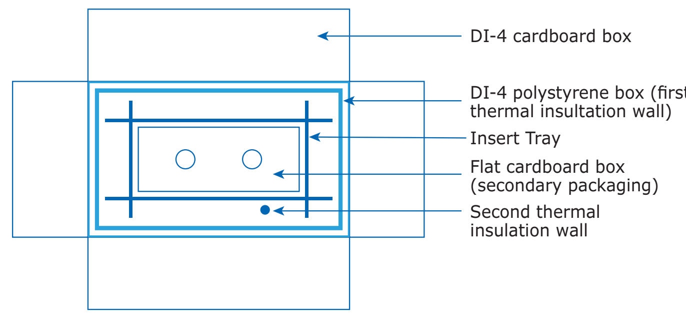


3. After placing the secondary flat box on the dry ice bottom layer, the DI-4 box is filled with dry ice. Then, a polystyrene lid is added and the DI-4 box is closed. The DI-4 is ready for shipping. A logo with directional arrows is visible on the DI-4 cardboard to ensure that shipment is performed in the right position



1.2.1.2 Packaging top view details

The secondary flat cardboard box containing the product is placed in the middle of the insert.



The purpose of the insert trays is to keep the flat cardboard box in the middle of the DI-4 box.

The insert trays also bring an additional thermal insulation:

- the first thermal insulation wall is composed of the polystyrene box of the DI-4
- the second thermal insulation wall consists of the outer cavities of the insert filled with dry ice

Different tests pointed out that the dry ice located in the 4 corner cavities of the insert tray sublimates the fastest. This is linked to the fact that these 4 corners cavities have directly 2 sides out of 4 in touch with the outer polystyrene wall. The dry ice of the lateral insert tray cavities, as well as those above the secondary flat cardboard box, sublimate more slowly. The dry ice placed under the secondary flat cardboard box sublimates the slowest.

1.2.2 Standard conditions of shipment

The packaging described previously is used as a standard condition for the transportation of Ultra-Freeze products from European distribution center in Strasbourg, France to the customer site.

2 EXPERIMENTAL CONDITIONS

2.1 Methods

2.1.1 Methods for thermal performance evaluation of the ultra-freeze shipment packaging

The thermal efficiency of the product packaging has been challenged in two ways:

- Simulation of worst case shipping temperatures with temperature cycling in a climatic chamber
- Real shipping from distribution center (Strasbourg, France) to Europe and North America

The purposes of the tests were:

- To verify if the packaging can maintain a stable ultra-low temperature in the central cavity
- To measure the temperature in the DI-4 box during box storage and shipping simulation
- To define the maximum number of secondary flat cardboard box that could ship together in the packaging while ensuring surrounding in dry ice during 5 days
- To verify the quantity of dry ice remaining in the main box after 5 days
- To control if there is still dry ice remaining on and under the secondary flat cardboard box after 5 days
- To control the temperature at different locations in the box

Probes installation:

- For each DI-4 box, a temperature sensor (data logger) was placed inside the flat cardboard box (secondary packaging) to monitor and register the temperature at which the frozen product is exposed during the transportation simulation. Temperature was recorded every 2 minutes

Several controls were performed on new packaging:

- Several configurations with multiple flat cardboard boxes (secondary packaging) in one DI-4
- Weighing of the DI-4 box before and after the test to define the quantity of dry ice remaining in the DI-4 box

The packaging evaluation was performed:

- At room temperature
- In climatic chamber. The whole package was placed in calibrated climatic chambers to mimic seasonal temperatures, one summer cycle (worst case for a product shipped under dry ice), in compliance with regulation NF S99-700 (Isothermal packaging and packaging coolers for products of health – method of qualification of the thermal performances). Temperature was read for each test

  A 5-days cycle (120h) was applied to the package installed in a dedicated climatic chamber. This duration is considered as a worst case since the average transit time for a dry ice shipment ex-distribution center (Strasbourg, France) is 24 to 48 hours in Europe and 3 days in North America.

  The cycle program applied to packages included both maximum and minimum temperatures described in the two standards:

  – Maximum temperature: 40 °C

  – Minimum temperature: 22 °C

  Only summer conditions were simulated as product is not sensitive to low temperatures when shipped in dry ice (no need to protect product against freezing).

Temperature cycle profile

Cycle 1	Time	3 h	4 h	9 h	8 h	4 h	3 h	9 h	8 h
	T°	22 °C	28 °C	22 °C	28 °C	40 °C	28 °C	22 °C	25 °C
Cycle 2	Time	3 h	4 h	9 h	8 h	4 h	3 h	9 h	8 h
	T°	22 °C	28 °C	22 °C	28 °C	40 °C	28 °C	22 °C	25 °C
Cycle 3	Time	3 h	4 h	9 h	8 h	4 h	3 h	9 h	8 h
	T°	22 °C	28 °C	22 °C	28 °C	40 °C	28 °C	22 °C	25 °C

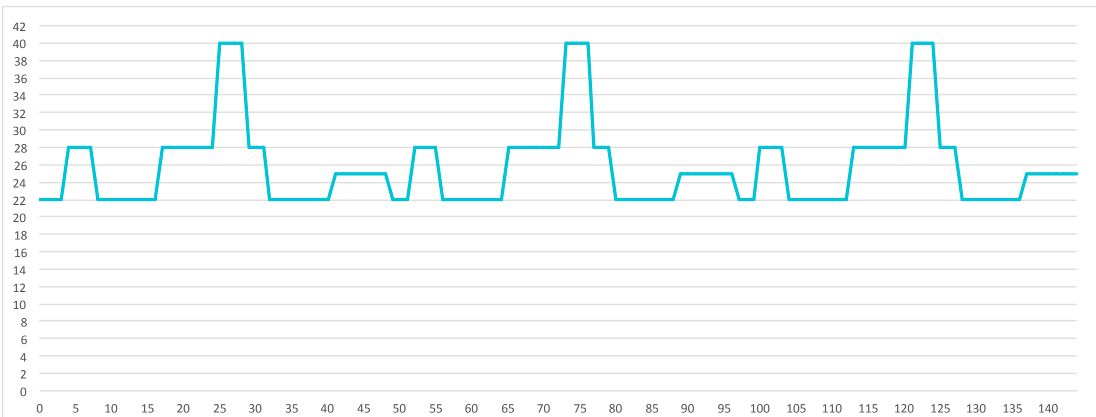


Figure 1. Temperature variations applied to the packages in climatic chamber

Note: This shipment simulation was focusing on the thermal capacity of the packages. It does not include product movement within packages due to handling or vibrations caused by the shipment.

Package preparation:

- Each package was prepared according to the procedure described previously (chapter 1.2.1)

  An insert tray was placed inside the DI-4 box. A first dry ice layer was put at the bottom in the DI-4 box.

  The product was placed in the secondary flat cardboard box(es). These secondary box(es) were positioned in the central cavity of the inserts on the dry ice layer. Dry ice was added on top of the secondary flat box(es) so that samples were properly covered and the DI-4 box was completely filled.

2.1.2 Method for Functional resistance evaluation of the packaging (ASTM D4169)

2.1.2.1 Protocol and Method

Tests were done at certified subcontractor according to ASTM D4169, which represents worst case shipping corresponding to air freight + road transportation:

- version 2016,
- distribution cycle 13
- severity II

After these packaging tests, each product was inspected to check if the packaging tests had an impact on the characteristics of the contained product.

ASTM D4169 DC13 level 2

Test protocol	Ultra-freeze DI-4 Box
Climatic conditioning 24 hr. at 23 °C + 50 % RH	N/A for dry ice
Drop test on 2 faces + 2 ridges + 2 corners	From height 305 mm
Dynamic compression	At 2132 N
Low frequency vibrations	40 min at 4 Hz
Depression	1 hr. at 59,5 kPa
Random vibrations	Profile « Truck »
Random vibrations	Profile « Air level II »
Localized impact	5.4 joules on the 6 faces
Drop test on 3 faces + 2 ridges + 1 corner	H = 305 + 710 mm

2.1.2.2 Material

The ASTM tests were performed on three boxes; the products were shipped in DI-4 packaging. As the current shipping is under express shipment (within 5 days), the preconditioning phase of the ASTM standard was skipped.

Important note: The boxes were shipped to the subcontractor, via express shipment, without overpackaging, not on a pallet. This is already a worst case, as the boxes are handled/shipped prior to the packaging simulation tests.

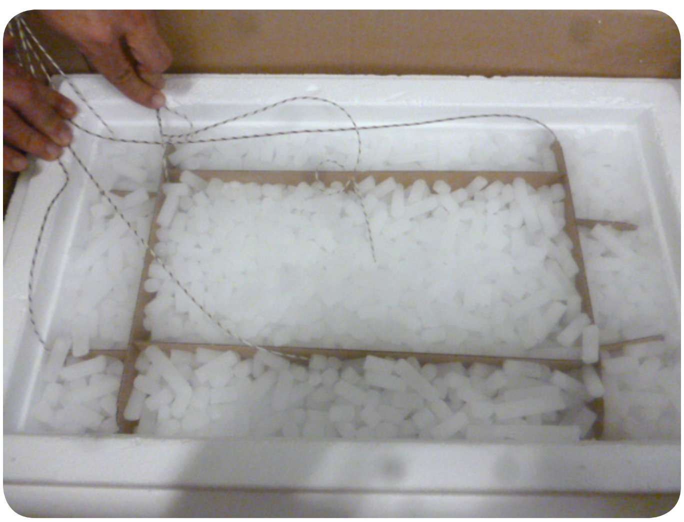
3 RESULTS

3.1 Thermal performance of the ultra-freeze shipment packaging

3.1.1 Packaging evaluation at room temperature

3.1.1.1 Description of the test

The test was performed with a DI-4 box containing several secondary flat cardboard boxes. These secondary flat cardboard boxes were stacked in the central cavity of the insert. In addition to the data logger, several probes were located at different places in the box, to have a whole picture of the temperature for 5 days.



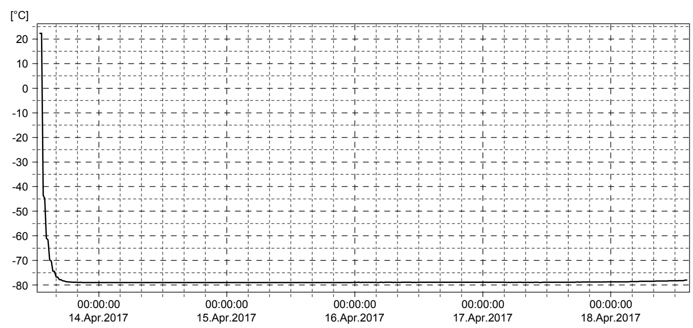
Each flat cardboard box was containing frozen products, the data logger was added in the top box.

The data logger and the probes were placed as following:

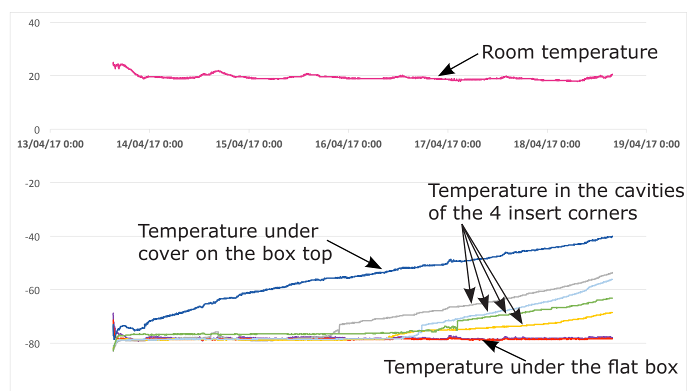
- Data logger inside the upper flat box (worst case position due to higher sublimation of dry ice)
- Probe 1 under the stacked flat cardboard boxes (between the dry ice and the lower flat cardboard box)
- Probe 2 on the top of the stacked flat cardboard boxes (between the upper flat cardboard box and the dry ice)
- Probe 3-4-5-6 inside each corner alveolus (in the dry ice approximately halfway up) to measure the evolution of the temperature in most sensitive places to variation of the temperature
- Probe 7 fixed to the upper part of the insert on the central cavity just below the box polystyrene cover to measure the evolution of the surrounding air temperature inside the box (probe very quickly out of contact with the dry ice)
- Probe 8 in the room where the DI-4 box was stored to measure the room temperature

3.1.1.2 Results

Data logger result and the monitoring of the 8 probes are the following:



Data logger temperature curve (inside the upper secondary flat box)



The 8 probes temperature curves

3.1.1.3 Interpretation

- After 5 days at room temperature, some dry ice is remaining under and above the stack of flat cardboard boxes. Thanks to the insert allowing several compartment and thermal insulation, the dry ice is not sublimated and allows a stable temperature on the top and under the stack of flat cardboard boxes
- The data logger was placed into the upper flat cardboard box (worst case). The temperature is stable for 5 days

- The temperatures corresponding to the probes located in the 4 corners raised progressively because the probes were not any more in contact with dry ice
- The temperature corresponding to the probe n°7, just below the DI-4 box cover, increased quickly due to the fast sublimation of dry ice on the top. The probe was not any more in contact with dry ice
- The measurement shows a stable temperature corresponding to the dry ice temperature when it is remaining

This experiment shows the importance of flat cardboard boxes to remain surrounded by dry ice through shipment. The temperature of the products is stable as long as there is dry ice, which is the case up to 5 days. The packaging meets the expectations in static mode when stored 5 days at room temperature around 20 °C.

3.1.2 Packaging evaluation in climatic chamber with 2 flat cardboard boxes

3.1.2.1 Description of the test

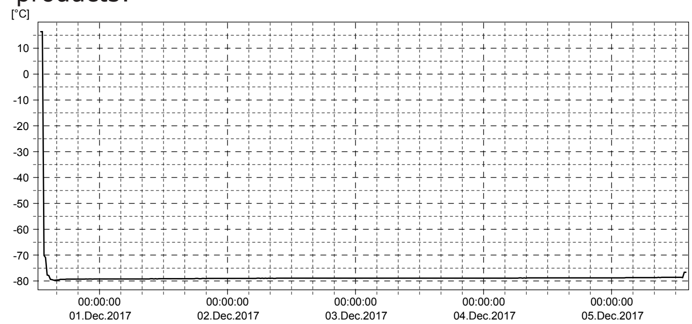
The test was performed with a DI-4 box containing 2 flat cardboard boxes for 5 days in climatic chamber according to a seasonal summer cycle (worst case, see details in chapter 2.2.1). These secondary flat cardboard boxes are stacked in the central cavity of the insert.

DI-4 + 2 flat cardboard boxes	Before climatic chamber			After 5 days of climatic chamber		
	Empty box	Box + dry ice	Dry ice total	Box + dry ice	Dry ice total	Dry ice on the top of the first flat cardboard box
Weight (kg)	2	25	23	10.3	8.3	1.8

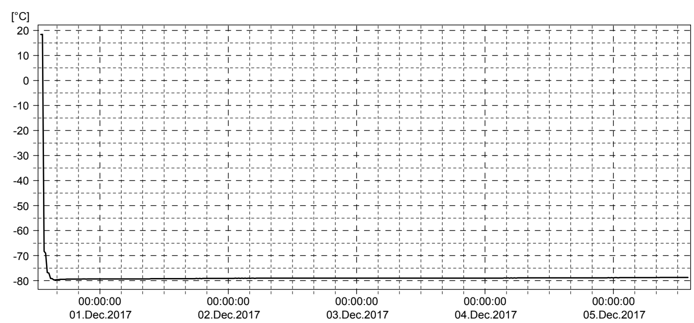
3.1.2.2 Results

Result of the DI-4 with 2 flat cardboard boxes:

Data logger showed a very stable temperature inside the 2 flat cardboard boxes containing the frozen products:



First flat cardboard box temperature curve



Second flat cardboard box temperature curve

3.1.2.3 Interpretation

Dry ice is remaining under and on the top of the 2 flat cardboard boxes placed in the central cavity of the insert trays. The temperature is stable for a 5-day summer cycle and the remaining dry ice is sufficient to maintain product at dry ice temperature (around -78 °C, -109 °F).

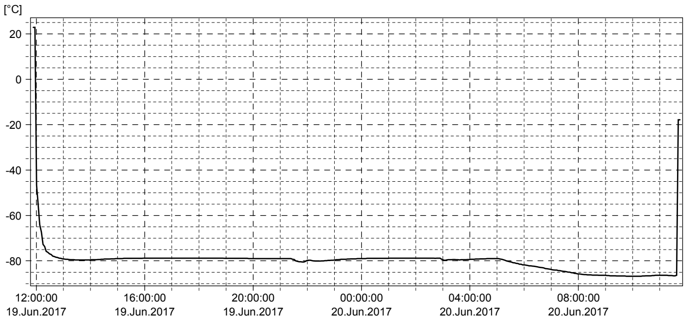
3.1.3 Europe shipment case

3.1.3.1 Description of the test

A parcel was sent to Darmstadt, Germany. The preparation of the package was done by MilliporeSigma according to the procedure described previously in this document and shipped with express service.

3.1.3.2 Packaging comments

After 1 day, the parcel was distributed to Darmstadt, Germany.



Temperature curve registered with data logger

The data logger registered the temperature inside the secondary cardboard box. Product was well stored at -80 °C or below. The peak at the end of the transportation corresponds to the opening of the box.

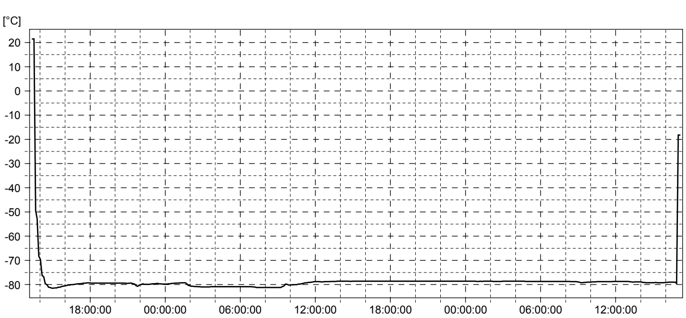
3.1.4 US shipment case

3.1.4.1 Description of the test

A parcel was sent to Burlington in the USA. The preparation of the package was done by MilliporeSigma according to the procedure described previously in this document and shipped with express service.

3.1.4.2 Packaging comments

After 2 days, the parcel was distributed to Burlington USA. The data logger registered the temperature. The product was well stored at -80 °C. The peak at the end of the transportation corresponds to the opening of the box.



Temperature curve registered with the data logger

3.2 Results for functional resistance of the packaging (ASTM D4169)

3.2.1 Preparation, shipment and reception of the DI-4 boxes at a packaging tester company

The aim was to ship the boxes to subcontractor and have them tested within 5 working days, in order to have the most dry ice in the boxes, thus having the heaviest weight, and thus being in the most unfavorable case for shipment test: the heavier the box is, the more stringent the drop tests are.

3.2.2 Test protocol, results and observations

The controls and tests at the subcontractor consisted in:

1. **Visual control of the packaging and weighing at reception:** some traces of bumps were observed on the boxes
2. **Climatic conditioning:** No preconditioning was applied to the boxes, as they contained dry ice
3. **Drop test from height= 305 mm:** Drop test done on several box faces, ridges and corners
    1. Face 1
    2. Ridge 3-5
    3. Ridge 2-3
    4. Corner 2-3-5
    5. Corner 3-4-6
    6. Face 3

    Observation: corners 2-3-5 and 3-4-6 slightly warped on the 3 boxes
4. **Dynamic compression:** applied force (depends on box size and weight): 2132 N: no visual defect
5. **Low frequency vibrations (US):** move of 1 inch, frequency of 4.1 Hz for 40 min: no visual defect
6. **Depression:** simulation of vacuum for 1 hr. (14000feet +/- 1000 ft.), pressure 59.5 kPa (ASTM D6653-01): no visual defect
7. **Random vibrations:** vertical random vibrations following ASTM D4169-16 profile truck), on 3 faces, for 60 min: no visual defect
8. **Random vibrations:** vertical random vibrations following ASTM D4169-16 profile air level II), on 3 faces, for 120 min: no visual defect
9. **Localized impact with impact energy of 5.4 joules, on the 6 faces of each box:** the box shall not move back or tilt. Result: perforation of the cardboard on the 6 faces of the 3 boxes
10. **Drop test from height = 305 mm, excepted last drop from 610 mm:**
    7. Ridge 2-6
    8. Face 4
    9. Face 6
    10. Corner 1-4-6
    11. Ridge 1-4
    12. Face 3

    Observation: corners 1-4-6 slightly warped on the 2 boxes

3.2.3 Conclusion

No specific deterioration of the overpackaging that could hinder its handling or stability for stacking was observed.

As the product contained was integer, the packaging successfully passed the test program.

4 CONCLUSION

4.1 Thermal performance of the ultra-freeze shipment packaging

The tests described in this protocol show the thermal performance of a new concept of packaging composed of:

- Secondary cardboard boxes, which are placed directly in the dry ice, in the center cavity of the insert tray
- An insert tray, which is placed into the DI-4 shipment box to ensure that the secondary cardboard boxes are adequately positioned and covered with dry ice throughout transit time

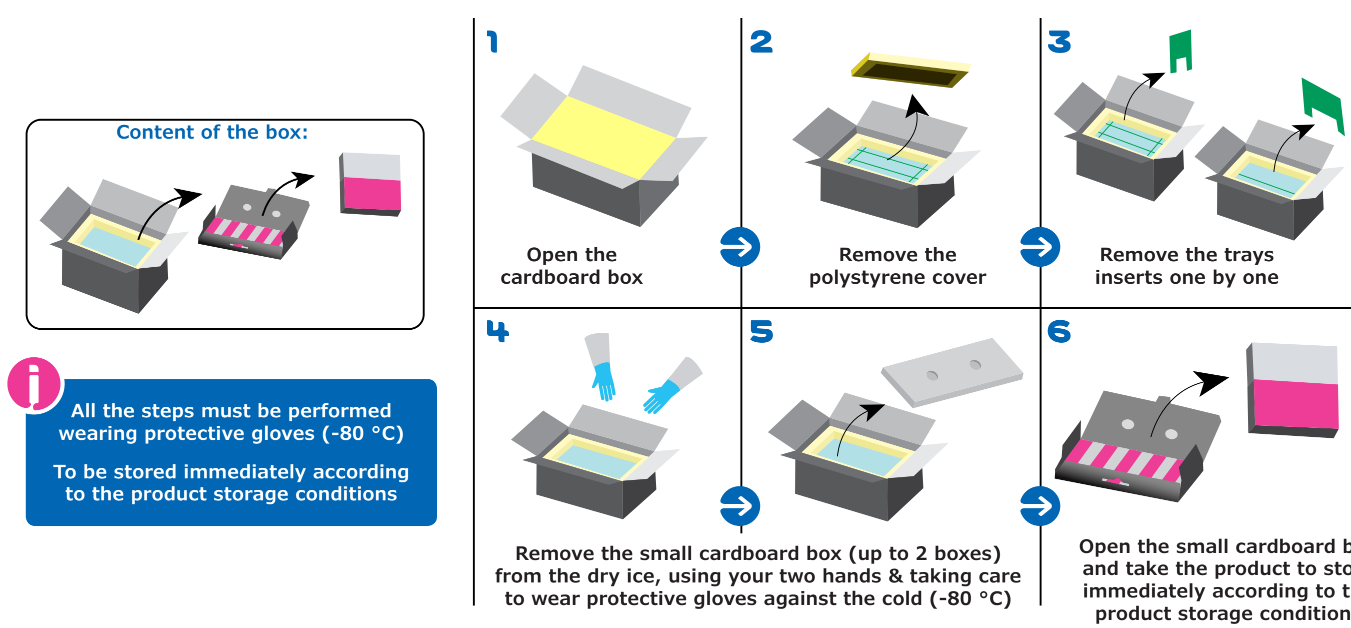
The characteristics of the packaging are described as following:

- The packaging can maintain a stable dry ice temperature for 5 days with two secondary flat boxes
- After 5 days, the remaining dry ice is sufficient to guarantee a stable temperature for the frozen product. The insert trays allow ensuring the secondary flat cardboard box is adequately positioned and covered with dry ice

A standard operating packaging procedure is used routinely by the distribution center (Strasbourg, France).

A quick guide is available for the customer to unpack correctly the frozen product. It is placed on the top of the DI-4 box during 6 months after implementation of the new solution, and will be available for customers on our website.

Standard operation procedure for unpacking



4.2 Functional resistance of the packaging (ASTM D4169 packaging)

The objectives of the present tests were to verify the ability of the packaging to protect the final product from any damage that would prevent the product use, and to avoid any safety risk for the operator:

- There is no specific deterioration of the packaging that could hinder its handling or stability for stacking; there is no risk for safety: no spilling of dry ice
- The content is integer
- The DI-4 packaging successfully passed the test program

The DI-4 packaging can be used for routine shipping of Ultra-Freeze Product.

Notes

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