Version G3.1

Novartis Technical Operations

# Novartis Leukapheresis Reference Manual

## CAR-T

**(KYMRIAH/Tisagenlecleucel/CTL019 and clinical CAR-T cell products)**

## Version G3.1

NOVARTIS

Leukapheresis material provides the starting material for CAR-T cell product manufacturing. This Leukapheresis Reference Manual details requirements and guidance for successful collection, cryopreservation, and shipping of leukapheresis material.

Following the steps in this Leukapheresis Reference Manual closely is necessary to prevent delay in product manufacturing.

Ensure that you are complying with local quality standards and the Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement.

Please contact Novartis Cell Therapy Operations with any questions.

**The Novartis Customer Service Center may provide additional assistance:**

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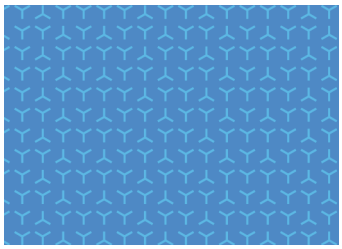

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## Process Overview

**LEUKAPHERESIS COLLECTION** › **CELL PROCESSING** › **PACKING AND SHIPPING**

## Guide to Icons

CellChain™ (Novartis Online Portal)

Important Information

Chain of Identity (COI)

Specifications

Time-Sensitive Process

Temperature Control/ Monitoring

Documentation Requirements

Communication with Novartis

Note: **All items specific to clinical trials are indicated in green.**

# Definitions

- **Apheresis collection unit:** Location where peripheral blood mononuclear cell (PBMC) leukapheresis material is collected
- **Cell processing laboratory:** Location where PBMC leukapheresis material is processed and cryopreserved
- **Chain of Identity (COI):** The detailed tracking and verification of a patient's cells and data throughout each step of the process, from cell collection and processing to product manufacturing and finished product administration
- **Collection Date/Time:** The date and time of the **end** of the leukapheresis collection
- **Cryopreservation Date/Time:** The date and time at which the cryoprotectant (dimethyl sulfoxide [DMSO]) is added to the leukapheresis material
- **Dewar:** A double-walled flask used to hold liquids at cryogenic temperatures
- **Dry vapor "shipper":** A container designed for the safe transportation of biological samples at cryogenic temperatures. It consists of two main components:
  - A protective outer shipping container
  - An enclosed dewar charged with liquid nitrogen (within the dewar, the liquid nitrogen is absorbed in a foam retention system)
- **Leukapheresis material:** A patient's autologous non-mobilized PBMCs collected via apheresis
- **Total blood volume (TBV):** Total volume of blood circulating in a patient, calculated based on the height, weight, and gender of the patient

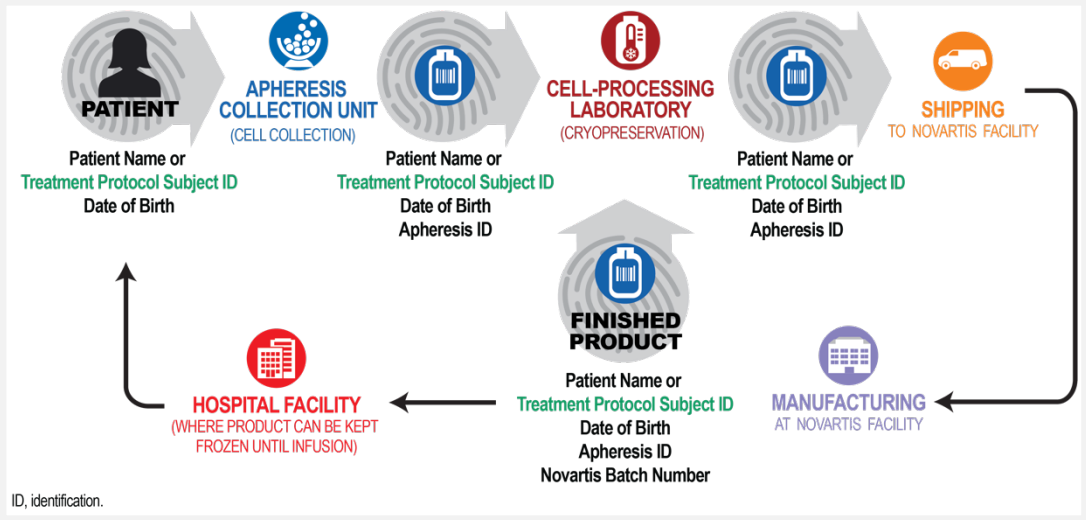
# Chain of Identity

Autologous product requires that cells collected from a patient be infused into the same patient after product manufacturing. The detailed tracking and verification of all patient materials and data are critical throughout each step of the process to ensure Chain of Identity (COI). Important instructions for maintaining COI are presented in this section and throughout this Leukapheresis Reference Manual.

## *Patient Identifiers*

Unique patient identifiers are used at each step in the process to accurately identify patient material. Figure 1 details the patient identifiers used at each step.

**Figure 1. Patient identifiers used in the CAR-T cell process[a]**




## *Chain of Identity Definitions*

- **Patient name:** Patient's first and last names in Roman characters (initial of middle name is optional)
- **Date of birth:** Patient's full date of birth. Date format may be specified
- **Apheresis ID:** Unique identification number for the specific patient donation (specific collection event). The Apheresis ID may be a:
    - Institutional assigned Apheresis ID: A unique identification number assigned locally by the hospital/apheresis collection unit that identifies the specific patient donation (specific collection event)
    - Donation Identification Number (DIN): A unique identification number assigned by the site using International Society of Blood Transfusion (ISBT)-128 labeling process for each collection event
    - Single European Code (SEC): A unique identification number assigned for each European blood/tissue donation (specific collection event)

  ***Note:*** *European sites must use a SEC or DIN as the Apheresis ID to meet European Union Directive requirements.*
- **Novartis Batch Number:** A unique identification number assigned to each product being manufactured by Novartis. The Batch may include more than one leukapheresis collection from the same patient
- **Treatment Protocol Subject ID:** A unique patient identifier assigned by Novartis to each patient after enrollment in the clinical trial protocol

[a]The labeling guidance for countries in Europe and other regions of the world may vary due to local data privacy laws. The local data privacy regulations will be followed for the clinical trials. Please contact Novartis Cell Therapy Operations for questions regarding region-specific labeling.

# CLT019/Tisagenlecleucel Leukapheresis Material Specifications

## Chapter Purpose

This chapter details requirements for timing of leukapheresis, leukapheresis material specifications, and guidance on collection cell counts. This section is about leukapheresis material specifications for CTL019/tisagenlecleucel; for T-Charge™ specifications, please refer to Appendix 4.

## Requirements for Timing of Leukapheresis Collection

- ✓ Cryopreserved leukapheresis material collected more than 30 months before the start of product manufacture date may **NOT** be shipped to Novartis without prior approval by Novartis

## Summary of Leukapheresis Material Specification Requirements

To be eligible for manufacturing, leukapheresis material must meet the specification requirements shown in Table 1.

For CTL019 clinical trials, please consult your Clinical Trial Protocol for potential additional specifications.

*(Continued on following page)*

**Table 1. Leukapheresis material specification requirements for CTL019/tisagenlecleucel**

| Specification | | Test Method/ Data Source | Acceptance Criteria |
|---|---|---|---|
| **Patient Infectious Disease Testing (Must be completed within 30 days of collection)[a]** | HBV, HCV, HIV | Autologous donor testing per institutional SOP using the health authority–approved test methods (Site-reported result) | Patient must not have HIV or active HBV or active HCV infection |
| **Collection Cell Counts** | Viable TNC | Per institutional SOP (Site-reported result) | ≥2 x $10^9$ TNCs[b] |
| | Total number of viable CD3+ T cells | Per institutional SOP (Site-reported result) | ≥1 x $10^9$ CD3+ cells[b] |
| | % T cells | Per institutional SOP[c] (Site-reported result) | ≥3% |
| **Cryobag** | Integrity | Visual inspection upon thawing at Novartis facility | Intact bag/hermetically sealed ports |

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SOP, standard operating procedure; TNC, total nucleated cells.
[a] Please see the Patient Infectious Disease Testing chapter for further details.
[b] Total cell counts are calculated by taking the sum of cell counts from multiple bags (if applicable).
[c] Percent (%) T cells will be autocalculated by CellChain™ based on site-reported data (Total CD3+ cells/TNC).

# Collection Cell Counts

## *Requirements*

To be eligible for product manufacturing, collected leukapheresis material must meet minimum specification value requirements for both CD3+ lymphocytes (total and percentage) and total nucleated cells (TNCs) as outlined in Table 1. Specification values are independent of the weight of the patient.

For clinical trials, please consult your Clinical Trial Protocol for potential additional requirements.

*Guidance*

- It is important to review the WBC differential of the collected leukapheresis material, including checking for the presence of tumor cells/blasts. Leukapheresis material containing high numbers of blasts or other non-CD3+ cells (eg, lymphoblasts, monocytes, etc.) may present challenges to T cell selection and cell expansion
    - It is recommended to report the WBC differential of the collected leukapheresis material, including the presence of blasts, in CellChain™
- Collections should target the minimum specification values, as described in Table 1 of this chapter
- Statistical analysis of product manufacturing by indication has shown a benefit to manufacturing when there are greater than 1 x $10^9$ CD3+ cells in the collected leukapheresis material
    - The optimal CD3+ cell collection target range for diffuse large B-cell lymphoma (DLBCL) is 1.5 x $10^9$ – 4 x $10^9$ CD3+ cells
    - When medically possible, it is preferred to perform an additional collection to achieve the optimal range for DLBCL
- Collections containing more than 4 x $10^9$ CD3+ cells may be shipped to Novartis. However, the additional cells may be greater than the number of cells needed for manufacturing. Cells above this threshold may be referred to as “Excess Leukapheresis Material” and may be requested to be split into two batches
- Rounding rules may be applied after collection, for example, 0.5 x $10^9$ CD3+ cells are rounded to 1 x $10^9$ and pass specification; however when medically possible, it is preferred to perform an additional collection to achieve the minimum specification values (Table 1)

*Contact Novartis Cell Therapy Operations if your result is less than 1.0 x $10^9$ or greater than 4 x $10^9$ CD3+ cells.*

# Patient Infectious Disease Testing

## Chapter Purpose

This chapter details requirements and guidance for patient infectious disease testing.

## Requirements for Patient Infectious Disease Testing

- ✓ To be eligible for product manufacturing, leukapheresis material may only come from patients who do not have, or did not have at the time of the collection:
  - Active hepatitis B virus (HBV) infection
  - Active hepatitis C virus (HCV) infection
  - Human immunodeficiency virus (HIV) infection

- ✓ If no other regulatory requirement applies, Novartis requires that specified testing for HBV, HCV, and HIV must be performed on patient blood drawn no more than 30 days prior to leukapheresis collection
  - If testing was completed more than 30 days prior to collection, the testing will need to be repeated
  - Testing completed on day of collection or any time *after* collection is acceptable

  ***Note:*** *For clinical trials, ensure that any infectious disease testing timing requirements in your Clinical Trial Protocol are followed.*

- ✓ Infectious disease testing must be performed according to local regulatory requirements. This may include:
  - Additional infectious disease tests not specified by Novartis
  - Specific timelines for performing infectious disease testing
  - Specific requirements for the test kits to use for testing
  - Specific requirements to ensure the laboratories are eligible to perform the infectious disease testing
  - Additional donor questionnaires

- ✓ Verification that these requirements have been completed must be entered into CellChain™

# Guidance for Patient Infectious Disease Testing

Interpretation of infectious disease test results is the responsibility of the medical institution and medical professionals treating the patient.

Novartis considers a Nucleic Acid Test (NAT), commonly performed by polymerase chain reaction (PCR), to be an appropriate confirmatory test to determine if there is an HIV infection or active HBV/HCV infection. NAT results would supersede serological testing results for the determination of manufacturing eligibility.

*Guidance for Interpretation of Hepatitis B Virus (HBV) Test Results*

**Table 1. Guidance for determining manufacturing eligibility based on HBV serologic test results**

| Test type | Results | | | | |
|---|---|---|---|---|---|
| HBsAG | + | – | – | – | – |
| HBcAB | Any | + | + | – | – |
| HBsAB | Any | – | + | + | – |
| ***Manufacturing Eligibility*** | *Not Eligible* | *Not Eligible* | *Eligible* | *Eligible* | *Eligible* |

HBcAB, hepatitis B core antibody; HBsAB, hepatitis B surface antibody; HBsAG, hepatitis B surface antigen.

Further guidance on interpretation of HBV test results may also be found in the reference below:

- Centers for Disease Control and Prevention. Interpretation of hepatitis B serologic test results. https://www.cdc.gov/hepatitis/hbv/pdfs/serologicchartv8.pdf

*Guidance for Interpretation of Hepatitis C Virus (HCV) Test Results*

An HCV antibody test can determine if a patient was exposed to HCV but cannot assess if an HCV infection is active. A nucleic acid test (NAT) for HCV RNA can be used to determine if an HCV infection is active.

A NAT/PCR test may be conducted as a follow-up test after antibody testing to confirm patient HCV status, or it may be performed as an initial test.

**Table 2. Guidance for determining manufacturing eligibility based on HCV NAT/serologic test results**

| Test type | Results | | | | |
|---|---|---|---|---|---|
| HCV AB | (Any) | + | + | − | (Not tested) |
| HCV NAT/PCR | + | (Not tested) | − | (Not tested) | − |
| ***Manufacturing Eligibility*** | *Not Eligible* | *Not Eligible* | *Eligible* | *Eligible* | *Eligible* |
| *Rationale* | *(Active infection)* | *(Presumptive active infection)* | *(Resolved or past infection)* | *(No infection)* | *(No infection)* |

AB, antibody; HCV, hepatitis C virus; NAT, nucleic acid test; PCR, polymerase chain reaction.

Further guidance on interpretation of HCV test results may also be found in the reference below:

- Centers for Disease Control and Prevention. Interpretation of results of tests for hepatitis C virus (HCV) infection and further actions. https://www.cdc.gov/hepatitis/HCV/PDFs/hcv_graph.pdf

*Guidance for Interpretation of Human Immunodeficiency Virus (HIV) Test Results*

- If a patient is previously assessed as HIV+, the patient is not eligible for product manufacture, regardless of whether the viral load on antiretroviral therapy is detectable or undetectable via NAT/PCR test
- If the patient is previously understood to be HIV− (not on antiretroviral therapy), but antigenic test results are positive, a NAT/PCR test can be performed to assess if antigenic test results are false positive. A NAT/PCR negative result indicates a false positive antigenic test result, and the patient is eligible for product manufacture

**Please contact Novartis Medical for additional information on patient infectious disease testing as needed.**

# Precollection: Determining Patient Readiness for Leukapheresis

## Chapter Purpose

This chapter provides medical recommendations for determining patient readiness for leukapheresis, in regard to current and prior therapies and the current medical condition of the patient.

Medical decisions related to patient treatment are the responsibility of institutional medical professionals. The recommendations included in this chapter are intended to provide information that may be valuable in these decisions.

For clinical trial patients, also follow requirements in your Clinical Trial Protocol when determining their eligibility and readiness for leukapheresis.

Please contact Novartis Medical with any questions related to this chapter.

## Introduction

Determining patient readiness for leukapheresis should be coordinated with the prescribing/responsible physician and carried out per institutional procedures before initiating collection.

To optimally time leukapheresis collection, attention should be given to the patient's:

- Initial health assessment
- Current and previous therapy timing
- Peripheral blood counts (eg, absolute lymphocyte count, CD3+ count, hematocrit)
    - ***Note:*** *Refer to the Leukapheresis Collection chapter for guidance on peripheral blood testing*

## Informed Consent for Clinical Trials

For sites performing leukapheresis as part of a study protocol, leukapheresis may only be performed after patient consent has been obtained.

# Initial Health Assessment

Before collection, patients should undergo a health assessment to ensure readiness for leukapheresis. Patient evaluation and documentation should be performed according to institutional procedures.

### *Requirements*

Refer to the chapters on CTL019/Tisagenlecleucel Leukapheresis Material Specifications and Patient Infectious Disease Testing and Appendix 4: T-Charge™ Leukapheresis Material Specifications for additional information.

Patients with an acute infection (bacterial, viral, or fungal) or associated positive blood culture (patient blood, catheter tip, etc.) within seven days prior to cell collection should not undergo leukapheresis collection.

Patients with a positive blood culture or signs of infection should complete a full course of anti-infective therapy before collection.

If there are any signs/symptoms of infection, pre-collection or within 48 hours post-collection (eg, temperature, positive blood culture) in the patient, or contamination of the venous access catheter, please notify Novartis Cell Therapy Operations.

*Guidance for Asymptomatic Viral Infections*

There are no data or information related to the potential impact of asymptomatic viral infections, such as COVID-19 or Zika, on the patient's leukapheresis material, product manufacturing, administration of lymphodepleting chemotherapy, infusion of finished product, or post-infusion outcomes, including efficacy and/or potential adverse events.

Following infectious disease testing, patients with any identifiable viral infections should be asymptomatic, should not be infectious, and should have completed a full course of treatment prior to leukapheresis collection when applicable and clinically appropriate. Given the lack of information on asymptomatic viral infections, the physician will communicate the potential risk to the patient and obtain patient consent for leukapheresis collection.

Please contact Novartis Medical for additional information on patient infectious disease testing as needed.

# Current and Previous Therapy Timing

For optimal product manufacturing success, collection of leukapheresis material should be timed with any additional therapies the patient is receiving or may have received. Documentation of a patient's medication history must be performed according to institutional procedures. Based on patient consent, Novartis may request to review the patient's medical history.

*General Guidance*

Novartis recommendations for drug washouts prior to leukapheresis are guidance only, and the patient's condition and disease status should also be considered when determining washout timing.

A washout period of 5 half-lives is adequate for drug clearance, but effects of some drugs on T cells may persist after drug clearance.

For therapies that are not defined or if half-life is short (eg, <24 hours), consider the following:

- Half-life and pharmacokinetics of the drug
- Effect of the drug/agent on T cell fitness and/or CD19 expression
- Potential for lymphopenia

If you have questions about these recommendations, please contact Novartis Medical.

*Recommendations on Washout Timing Prior to Leukapheresis*

Recommendations for cessation of therapies prior to leukapheresis are shown in Figure 1 on the following page. For further details on washout recommendations, please refer to Novartis' *"Guidance for Therapy and Drug Washout Prior to Leukapheresis for Tisagenlecleucel Manufacture."*[1]

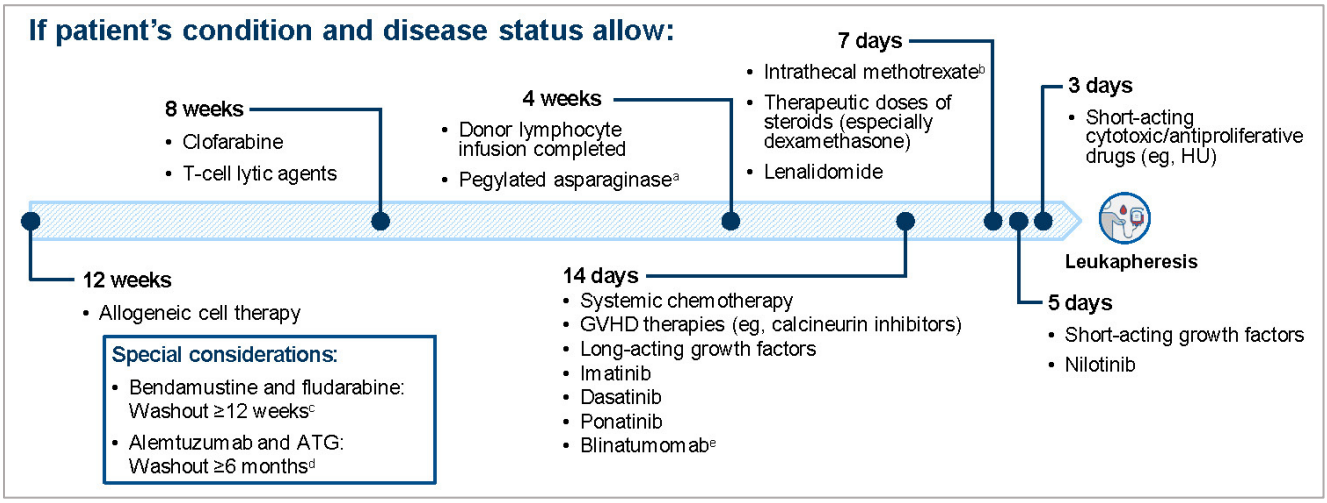
Note that recommendations are current as of the date of publication of the Leukapheresis Reference Manual. Please refer to Novartis Medical for any updates.

Specific therapy timing requirements found in your Clinical Trial Protocol must also be followed.

*(Recommendations continued on following page)*

## *Recommendations on Washout Timing Prior to Leukapheresis (continued)*

**Figure 1. Recommended timing to stop therapies prior to leukapheresis[1]**




ATG, antithymocyte globulin; CAR, chimeric antigen receptor; GVHD, graft-vs-host disease; HU, hydroxyurea; pALL, pediatric acute lymphoblastic leukemia.

[a]In the CASSIOPEIA trial (NCT03876769) for pALL, the recommended washout period is 14 days.
[b]If indicated, intrathecal cytarabine can be given up to a day prior to leukapheresis. For an intravenous cytarabine dose <100 mg/m$^2$, a washout of 7 days is recommended; for a dose ≥100 mg/m$^2$, a washout of 14 days is recommended.
[c]For bendamustine and fludarabine, allow adequate washout and avoid use for ≥12 weeks prior to leukapheresis due to the potential long-term effects on T cells; however, there are limited data in the context of CAR-T cell therapy for these agents. Please refer to the Novartis Medical Guidance for further information.
[d]Alemtuzumab and ATG (T cell lytic agents): Allow adequate washout and avoid use for ≥6 months prior to leukapheresis and consider the potential prolonged effects of T cells. Please refer to the Novartis Medical Guidance for further information.
[e]Although blinatumomab half-life is short (~2 hours), it is recommended to washout 1 to 2 weeks prior to leukapheresis.

**Reference: 1.** Data on file. Guidance for Therapy and Drug Washout Prior to Leukapheresis for Tisagenlecleucel Manufacture. Novartis Pharmaceuticals Corp.

## *Guidance on Steroid Washout Prior to Leukapheresis*

- Systemic and therapeutic doses of corticosteroids (prednisone and dexamethasone) should be avoided, if possible, in the week prior to leukapheresis
- Physiological replacement doses of steroids are allowed—no washout required
    - Up to 12 mg/m$^2$/day hydrocortisone or equivalent in pediatric patients
    - Up to 40 mg/day hydrocortisone or equivalent in adult patients
- Topical or inhaled steroids for localized treatment of graft-vs-host disease (GVHD) are allowed—no washout required

## *Guidance on Polatuzumab*

- Polatuzumab is approved to be co-administered with bendamustine and rituximab, and the bendamustine washout period should be prioritized

# Leukapheresis Collection

## Chapter Purpose

This chapter defines the requirements for the non-mobilized peripheral blood mononuclear cell (PBMC) leukapheresis collection that will be used as starting material for product manufacturing, and it provides guidance for optimizing cell collection.

## Determining Patient Readiness for Leukapheresis

Refer to the chapters on CTL019/Tisagenlecleucel Leukapheresis Material Specifications and Patient Infectious Disease Testing and Appendix 4: T-Charge™ Leukapheresis Material Specifications for additional information.

Refer to the Precollection chapter of this manual for medical recommendations in regard to current and prior therapies and the medical condition of the patient.

## Guidance on Peripheral Blood Testing

Results of pre-leukapheresis peripheral blood testing may be used to estimate the minimum total blood volume (TBV) to process to achieve a successful collection.

*Collected leukapheresis material must meet the minimum specification for collected total nucleated cells (TNC), CD3+ cell counts, and CD3+% of TNC as outlined in the CTL019/Tisagenlecleucel Leukapheresis Material Specifications chapter and Appendix 4: T-Charge™ Leukapheresis Material Specifications of this Leukapheresis Reference Manual.*

Novartis recommends that if institutions have their own apheresis prediction algorithms based on CD3+ cell counts and collection efficiency, they may be used to predict the blood volume to be processed.

You may be asked to report peripheral blood testing results if performed.

For clinical trials, please also refer to your Novartis Clinical Trial Protocol for specific peripheral blood cell count requirements.

*Guidance on Calculating TBV to Process if Collection Efficiency Is Unknown*

If collection efficiency is unknown or not routinely utilized to target CD3+ collections, Table 1 below provides guidance on how much blood to process in order to meet leukapheresis material specifications.

**Table 1. Guidance on TBV to be processed based on peripheral blood counts of patients**

| If the absolute lymphocyte count (ALC) is: | And/or the CD3+ cell count is: | The patient's TBV to be processed is: |
|---|---|---|
| <100 cells/µL | <100 cells/µL | Consider 2 days of collections |
| <300 cells/µL | <150 cells/µL | 3 to 4x |
| >300 cells/µL | >150 cells/µL | 2 to 3x |
| Significantly high | Significantly high | Consider a smaller collection of 2x |

**Note:** If ALC is not equivalent to the CD3+ count, use the CD3+ count to estimate how much to process of the patient's TBV.

**If collection efficiency is known, please see the "Additional Notes" section at the end of this chapter for guidance on calculating the TBV to process.**

## Identity Verification and Collection Bag Labeling

### *Requirements*

- ✓ Follow institutional procedures for verification of the patient's identity
- ✓ Collection bag must be labeled with an affixed/attached label. **Do not write directly** on the surface of the bag
- ✓ Labels must meet local regulatory requirements per country regulations
- ✓ Collection bag labels must contain the following 3 mandatory identifiers:
  - Patient name or Treatment Protocol Subject Identification (ID)*
  - Patient date of birth*
  - Apheresis ID: Donation Identification Number (DIN), or Single European Code (SEC), or institutional assigned Apheresis ID

  ****Note:** For clinical trials, this may vary by country*
- ✓ Multiple collections on the same day must be documented and each must be labeled with a unique DIN/SEC/institutional assigned Apheresis ID
- ✓ Labeling accuracy must be verified by 1 qualified staff member using a validated process (eg, bar code reader) or by 2 qualified staff members

*Guidance*

Use of International Society of Blood Transfusion (ISBT)-128 labeling and computer-generated labels is preferred.

# Leukapheresis Collection

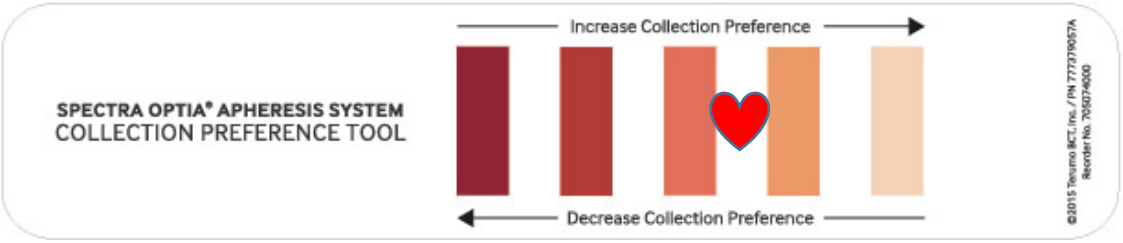
## *Requirements*

- ✓ The apheresis system and supplies must be approved for mononuclear cell (MNC) collection by your local health authority
  - Examples of apheresis systems include the Spectra Optia® (Terumo BCT, Inc), Fenwal Amicus® (Fresenius Kabi AG), Com.Tec® (Fresenius Group)
- ✓ Reagents must be of a grade suitable for human use, and no research-grade reagents may be used
- ✓ Anticoagulant citrate dextrose solution A (ACD-A) is required for anticoagulation during the apheresis collection
  - ACD-A plus heparin may be used for anticoagulation, if indicated, per institutional standard operating procedure (SOP)
  - Use of heparin alone is not permitted
- ✓ Conduct non-mobilized peripheral blood mononuclear cell leukapheresis collection
- ✓ Follow institutional guidelines for leukapheresis collection
- ✓ Process a volume of blood sufficient to obtain the minimum specification values of cells necessary
  - Multiple days of collection are permitted and may be needed to meet specifications
  - Note: In cases of multiple collections, only the Apheresis ID (DIN, SEC, or institutional assigned Apheresis ID) from the first collection will be present on the finished product label

*Guidance on Collection Procedure Optimization*

To optimize collections of T cells and to reduce red blood cell (RBC) and granulocyte cell types, consider the following:

- **Guidance when using the Spectra Optia®**:
  - Either the Spectra Optia® Continuous Mononuclear Cell Collection (CMNC) program or the Spectra Optia® Mononuclear Cell Collection (MNC) program may be used
  - The collect line should be maintained at a light salmon color, per the Spectra Optia® Apheresis System Collection Preference Tool




  - The default collect rate is 1.0 mL/min. For patients with lower absolute lymphocyte count (ALC) and CD3+ counts, monitor the collect line and consider modifying the collect rate to 0.8 mL/min
- **Guidance when using the Fenwal Amicus®**:
  - A maximum white blood cell (WBC) flow rate of 65 to 80 mL/min should be considered, and a red blood cell (RBC) offset of 6.8, and a MNC offset of 1.5 to start
  - Adjust the MNC and RBC offsets based on the color of the collect line, and maintain a light salmon color
- **Other Optimization techniques to consider:**
  - Answer alarms promptly
  - Evaluate access with Draw and Return access alarms
  - Process enough blood volume to meet apheresis specifications
  - Consider developing a processing prediction algorithm
  - Closely monitor the collect line
  - Monitor for clumping – adjust anticoagulant (AC) ratio as needed
  - Contact device manufacturer for further advice

Please contact Novartis Cell Therapy Operations to discuss collection optimization and strategies to prevent overcollection.

*Guidance on Collection in Smaller-Sized Patients*

Small-sized/pediatric patients weighing 25 kg or less can present specific collection challenges. Please see Appendix 1 (Leukapheresis in Small Patients) for additional guidance.

- For patients weighing <10 kg, consult with Novartis Cell Therapy Operations prior to collection since more than one day of collection may be needed

## Documentation of the Leukapheresis Collection

### *Requirements*

- ✓ Steps performed must be documented in institutional records following good documentation practices
- ✓ The following information must be documented:
  - Apheresis system used for collection
  - Volume of blood processed
  - Volume of leukapheresis material collected
  - Collection date/time (end time of leukapheresis collection)
- ✓ Information pertaining to materials and reagents used in the collection must be documented in institutional records. This information includes:
  - Manufacturer/supplier
  - Lot number
  - Expiration date

## Routine Data and Nonroutine Event Reporting

### *Requirements*

- ✓ CellChain™ must have data entry completed and approved as soon as possible/prior to shipment of leukapheresis material
- ✓ Follow any requirements in the applicable Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement for reporting of issues that may impact the leukapheresis material to Novartis. Examples of reportable issues include:

  - Leukapheresis collection-related serious adverse events (SAEs)
  - Potential contamination or cross-contamination, including a leak in the apheresis kit
  - Sterility failures
  - Chain of Identity (COI) breaches
  - Instrument malfunctions/failures

# Additional Notes

*Guidance on Calculating TBV to Process If the Collection Efficiency Is Known*

The TBV to be processed can be estimated based on available peripheral blood absolute CD3+ count, collection efficiency, and target CD3+ cell number. Equation 1 below provides an example calculation.

Cell collection should target the defined minimum specification values.

**Equation 1. Calculating estimated minimum total blood volume to be processed[a]**

$$\text{Estimated minimum total blood volume to be processed (L)} = \frac{\text{Target number of CD3+ cells to be collected}^{b}}{\text{Collection efficiency}^{c} \times \text{Peripheral CD3+ lymphocyte count (cells/µL)} \times 10^{6}\ \mu L/L}$$

[a] Units for each variable are listed in parentheses.
[b] Target the minimum collected CD3+ cell count specification or target a higher optimal collection as medically appropriate.
[c] Collection efficiency should be entered into the equation as a decimal (eg, 40%=0.40).

*Description of Equation Variables*

- Peripheral CD3+ lymphocyte count
  - This value can be obtained through prescreening of the patient's peripheral blood
- Collection efficiency
  - This represents the calculated percentage of target cells that are collected after being cycled through the apheresis machine
  - Average collection efficiency should be determined from historical samples collected on-site using the same machine

  ***Note:*** *It may take up to 10 collections to calculate the collection efficiency accurately.*

Collection efficiency can be calculated retrospectively using Equation 2.

**Equation 2. Calculating collection efficiency[a]**

$$\text{Collection efficiency (\%)} = \left[\frac{\text{Total CD3+ lymphocyte count in leukapheresis material (cells)}}{\text{Peripheral CD3+ lymphocyte count (cells/µL)} \times 10^{6}\ \mu L/L \times \text{Total blood volume processed (L)}}\right] \times 100\%$$

[a]Units for each variable are listed in parentheses.

# Leukapheresis Material Transfer and Testing

## Chapter Purpose

This chapter defines the requirements and recommendations for the transfer, storage, timing of cryopreservation, and testing of leukapheresis material collected for product manufacturing.

## Transfer, Storage, and Timing of Cryopreservation

### *Requirements*

- ✓ Transfer leukapheresis material to the cell processing laboratory in an appropriate container (ie, using a validated or temperature-monitored hard-sided insulated transport container), and document per institutional standard operating procedures (SOPs)
  - The appropriate transport container will vary depending on institutional policy and distance between the apheresis collection unit and cell processing laboratory

- ✓ Leukapheresis material stored for more than 4 hours must be held at 2°C to 8°C until cell processing is initiated
- ✓ Cryopreservation must be initiated within 24 hours from the end of the leukapheresis collection
  - Cryopreservation date/time is defined as the date and time that the cryoprotectant (dimethyl sulfoxide [DMSO]) is added to the leukapheresis material

*Guidance*

**It is highly recommended to cryopreserve as soon as possible after collection, or on the same day whenever possible.**

***Note:*** *Some clinical sites may be asked to provide Novartis with fresh collected leukapheresis material. In these cases, alternate instructions for packing and shipping will be provided.*

# Leukapheresis Material Testing

*Background*

Testing should be coordinated between the apheresis collection unit and the cell processing laboratory, as each test will need to be performed only once.

Leukapheresis material testing is used to determine if specification requirements have been met and to guide calculations and decisions as to the cell concentration and number of cryobags needed for cryopreservation.

**Note:** If minimum collected cell counts are not met, a second collection may be indicated. Please contact Novartis Cell Therapy Operations as soon as possible if a second collection is needed as this may impact the timing of the planned shipment of leukapheresis material to Novartis.

**Reminder:** To be eligible for manufacturing, leukapheresis material must meet the specification requirements, as shown in Table 1 for CTL019/tisagenlecleucel. (For T-Charge™ specification requirements, please refer to Appendix 4.) Please also see the CTL019/Tisagenlecleucel Leukapheresis Material Specifications chapter and Appendix 4: T-Charge™ Leukapheresis Material Specifications for additional guidance on collected cell counts.

**Table 1. Leukapheresis material specification requirements**

| Specification | | Test Method/ Data Source | Acceptance Criteria |
|---|---|---|---|
| **Patient Infectious Disease Testing (Must be completed within 30 days of collection)[a]** | HBV, HCV, HIV | Autologous donor testing per institutional SOP using the health authority–approved test methods (Site-reported result) | Patient must not have HIV or active HBV or active HCV infection |
| **Collection Cell Counts** | Viable TNC | Per institutional SOP (Site-reported result) | ≥2 x $10^9$ TNCs[b] |
| | Total number of viable CD3+ T cells | Per institutional SOP (Site-reported result) | ≥1 x $10^9$ CD3+ cells[b] |
| | % T cells | Per institutional SOP[c] (Site-reported result) | ≥3% |
| **Cryobag** | Integrity | Visual inspection upon thawing at Novartis facility | Intact bag/hermetically sealed ports |

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SOP, standard operating procedure; TNC, total nucleated cells.

[a] Please see the Patient Infectious Disease Testing chapter for further details.

[b] Total cell counts are calculated by taking the sum of cell counts from multiple bags (if applicable).

[c] Percent (%) T cells will be autocalculated by CellChain™ based on site-reported data (Total CD3+ cells/TNC).

*Contact Novartis Cell Therapy Operations if your result is less than 1.0 x $10^9$ or greater than 4 x $10^9$ CD3+ cells.*

### *Requirements*

- ✓ Testing (quality control) must be performed on the **fresh** leukapheresis material (prior to cryopreservation), not on thawed cryopreserved samples
- ✓ Prior to removing a sample of leukapheresis material for testing, the cell suspension must be gently mixed to ensure that a representative sample is tested
- ✓ All sampling must be performed aseptically to prevent contamination
- ✓ The following results must be generated and documented:
  - Complete blood count (CBC) with differential
  - Viable total nucleated cell (TNC) count
  - Viable CD3+ cell count
  - Total cell viability percentage

For clinical trials, please also refer to your Novartis Clinical Trial Protocol for additional testing that may be required.

***Note:*** *For patients with acute lymphoblastic leukemia (ALL), it is important that the CBC differential is capable of differentiating lymphoblasts. This can be accomplished with a manual differential, or some types of automated differential. This is important to ensure that appropriate adjustments are made to CD3+ flow cytometry results, as high blast counts can result in overestimated results being reported.*

*Guidance*

It is expected that test results for CD3+ cell count may not be available before cryopreservation. It is important to initiate the cryopreservation procedure while waiting for testing results.

Guidance for calculating cell counts is provided in the “Additional Notes” section at the end of the Leukapheresis Cell Processing chapter.

It is important that reported cell counts and volumes for the cryobags are accurate and appropriately account for volume removed for sentinel (QC) vials.

## Microbial/Sterility Testing

Microbial/Sterility testing of leukapheresis material is not required by Novartis for product manufacturing, but may be required by your institutional SOPs, and is strongly recommended by Novartis.

If microbial/sterility testing of the leukapheresis material is performed, it is recommended that sampling occur after addition of the cryopreservative (after the cryoformulation is complete) and no additional aseptic open processing steps will occur.

- ✓ Any positive results must be reported to Novartis as soon as possible per the notification time period in your approved Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement
- ✓ Leukapheresis material that initially tests positive for microbial contamination may not be shipped to Novartis unless an investigation is completed that invalidates the initial testing results

***Note:*** *Novartis finished product is tested for sterility.*

## Routine Data and Nonroutine Event Reporting

### *Requirements*

- ✓ CellChain™ must have data entry completed and approved as soon as possible/prior to shipment of leukapheresis material
- ✓ Follow any requirements in the applicable Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement for reporting of issues that may impact the leukapheresis material to Novartis. Examples of reportable issues include:
  - Potential contamination or cross-contamination
  - Sterility failures
  - Chain of Identity (COI) breaches
  - Instrument malfunctions/failures

# Leukapheresis Cell Processing

## Chapter Purpose

This chapter defines the requirements and recommendations for the cryopreservation of leukapheresis material collected for product manufacturing.

## Cell Processing

*Background*

Each institution will have its own procedures for cell processing, which may include steps for centrifugation, platelet washing, red cell depletion, and plasma reduction. Institutions should utilize their internal SOPs to the extent that they do not conflict with the requirements stated in this Leukapheresis Reference Manual. If a conflict is identified, it should be communicated to Novartis Cell Therapy Operations as soon as possible so that the differences can be evaluated.

Novartis recommends that cells be cryopreserved within the acceptable concentration range presented below. Cells cryopreserved lower than the acceptable concentration range may pose issues with analytical testing performed on sentinel (QC) vials. Cells cryopreserved higher than the acceptable range may aggregate upon thaw.

*Optimal target final white blood cell (WBC) concentration for cryoformulation:*

***1 x $10^8$ WBC/mL***

*Acceptable range:*

*0.5 x $10^8$ to 2.5 x $10^8$ WBC/mL (or 50 x $10^3$ to 250 x $10^3$ WBC/µL)*

It will be necessary to perform calculations to determine the processing steps needed to achieve the target final cryoformulation (final formulation with cells) concentration per bag. These calculations will guide processing steps (for example, volume reduction) and the total number of cryobags needed to freeze all cells. For guidance on calculating cell counts, please see the “Additional Notes” section at the end of this chapter.

The Novartis-preferred process is to reduce volume and add cryoprotectant solution in order to achieve the target cell concentration. See Appendix 4 for T-Charge™ volume specifications.

It is recommended to cryopreserve in at least 2 separate cryobags when the processing calculations permit.

In addition to cryobags that will be prepared, sentinel (QC) vials will also be filled with leukapheresis material at the same cryoformulation and WBC concentration.

## *Requirements for Cell Processing*

- ✓ Institutions must only use the processes defined and agreed upon with Novartis
- ✓ Equipment for cell processing must be established and qualified for use
- ✓ Open manipulation of the leukapheresis material must be performed using aseptic techniques in an International Organization for Standardization (ISO) Class 5 environment (for example, a class II biological safety cabinet)
- ✓ To prevent risk of cross-contamination, only one patient's leukapheresis material may be processed in a biological safety cabinet at one time

- ✓ Multiple collections for the same patient must be processed separately

### *Guidance on Cell Processing*

For best practices for cell processing/cryopreservation of leukapheresis material, please see the section later in this chapter.

For additional information on contamination control and environment monitoring, refer to *Novartis External Guidance: Contamination Control for Collection and Cryopreservation of Apheresis Material,* available from your Novartis Cell Therapy Operations Manager.

## *Requirements for Cryoformulation*

- ✓ Cryoformulation (final formulation with cells) must be approved by Novartis
- ✓ Cryoformulation must contain the following elements:
  - Dimethyl sulfoxide (DMSO)
  - Protein (or autologous plasma)
  - An electrolyte solution may also be required
- ✓ Cryoformulation must not contain nonhuman-derived additives (eg, bovine serum albumin) or starch and must be suitable for further manufacturing (approved for human use whenever possible)

*Guidance on Cryoformulation*

The following cryoformulations (final formulation with cells) are preapproved and do not need to be included in additional approval documentation:

- Dimethyl sulfoxide (DMSO): 5% to 10%
- Protein:
  - Human serum albumin (HSA): 2.5% to 10%
  - Autologous plasma: any percentage
  - Human AB serum: <5%
- Electrolyte solution:
  - Plasma-Lyte A, X-VIVO, Normosol or Normosol-R, saline
  - Not required if autologous plasma used as protein source

**Note:** Alternate cryoformulations need to be evaluated by Novartis and if approved should be documented in an approved Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement or other signed documentation from Novartis.

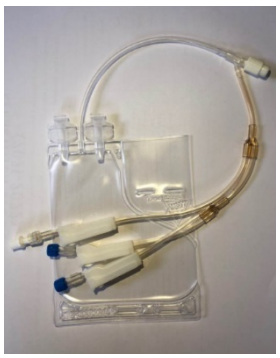
## *Requirements for Cryobags*

- ✓ Leukapheresis material must be cryopreserved in a cryobag approved by Novartis and must be approved by health authorities as a licensed medical device for cryopreservation
  - Cells cryopreserved in vials are not acceptable for manufacture
- ✓ Cryobag fill volume must not exceed the maximum fill volume recommended by the cryobag manufacturer
- ✓ Cryobags must fit into the Novartis-sized cassettes with the following dimensions:
  - *Standard cassette dimensions: 20.1 x 13.97 x 1.11 centimeters*
  - *Oversize cassette dimensions: 27.8 x 15 x 1 centimeters*
- ✓ A maximum of 10 cryobags may be sent to Novartis for manufacture

*Guidance on Cryobags*

The following examples of cryobags are preapproved by Novartis and do not need to be included in additional approval documentation:

- CryoStore™ Freezing Bags CS50, CS250, CS500, CS750 (OriGen Biomedical, Austin, Texas)
- CELL FREEZE® CF-50, CF-250, CF-500 (Charter Medical Ltd, Winston-Salem, NC)
- CryoMACS® Freezing Bags 50, 250, 500, 750 (Miltenyi Biotec, Bergisch Gladbach, Germany)
- EVA GSR2001AU (Macopharma, Mouvaux, France)
- F-050 (NIPRO, Osaka, Japan)



**Note:** Alternate cryobags will need to be evaluated by Novartis and if approved, should be documented in an approved Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement or other signed documentation from Novartis.

## *Requirements for Sentinel (QC) Vials*

- ✓ Sentinel (QC) vials must be 1- to 2-mL cryovials approved for storing cells at cryogenic temperatures
- ✓ Sentinel (QC) vials must be filled with ≥1 mL of leukapheresis material **with the same cryoformulation and WBC concentration as that in the cryobags**
- ✓ Collect up to 5 sentinel (QC) vials:
  - One (1) vial will be stored at your site per institutional SOPs at least until delivery of finished product
  - Two (2) to four (4) vials will be shipped with the cryopreserved material. Four (4) shipped vials are preferred, but it is acceptable to collect and ship fewer vials—at least 2 vials—in cases with low cell counts

***Note:*** *Additional vials may be required and possibly shipped to a different location for clinical trials. Refer to your Clinical Trial Protocol and/or central lab manual for a description of additional samples to be collected. Contact your Novartis Clinical Research Associate in case of questions.*

- ***In cases of low cell counts, the vials to the manufacturing facility should be prioritized***

# Cryobag and Sentinel (QC) Vial Labeling

## *Requirements*

- ✓ Cryobags must be labeled with an affixed/attached label prior to cryopreservation. Sentinel (QC) vials must be labeled legibly prior to cryopreservation. Do not write directly on the surface of the cryobag
- ✓ Labels must meet local and country regulatory requirements
- ✓ **Cryobag labels** must contain the following 3 mandatory identifiers:
  - Patient name or Treatment Protocol Subject Identification (ID)*
  - Patient date of birth*
  - Apheresis ID: Donation Identification Number (DIN), or Single European Code (SEC), or institutional assigned Apheresis ID

  ****Note:** For clinical trials, this may vary by country.*

  Each label must also contain a unique cryobag ID:
  - Cryobags must be sequentially identified (eg, 1 of 2, 2 of 2; A0, B0, C0)
- ✓ **Sentinel (QC) vial labels** must contain the following 2 mandatory identifiers:
  - Apheresis ID: DIN, SEC, or institutional assigned Apheresis ID
  - At least 1 additional patient identifier:
    - Patient name or Treatment Protocol Subject ID
    - Patient date of birth
    - Date of collection

- ✓ Labeling accuracy must be verified by 1 qualified staff member using a validated process (eg, bar code reader) or by 2 qualified staff members
- ✓ Patient name, Treatment Protocol Subject ID, and date of birth on labels must match exactly with patient identifiers in CellChain™
- ✓ The DIN/SEC/institutional assigned Apheresis ID entered into CellChain™ during data reporting must match that on the cryobag and sentinel (QC) vial labels

*Guidance*

Use of International Society of Blood Transfusion (ISBT)-128 labeling and computer-generated labels is preferred.

Additional labeling requirements, including use of Novartis-provided hangtags, may apply if ISBT-128 labeling is not used and/or to meet local country or regulatory regulations (eg, the SEC in the European Union). See Appendix 3 (Use of Novartis Hangtags) for additional details on use of hangtag labels.

# Cryopreservation

## *Requirements*

- ✓ Cryopreservation must be initiated within 24 hours from the end of the leukapheresis collection

- ✓ Multiple collections for the same patient must be cryopreserved separately
- ✓ Cryobag and sentinel (QC) vial labeling must be verified to confirm Chain of Identity (COI)

- ✓ Cryobags must be hermetically sealed prior to freezing
- ✓ Cryobags must be frozen using the metal cassettes provided by Novartis or using institutional cassettes or using freezing plates. **Avoid writing on metal cassettes**
- ✓ Sentinel (QC) vials must be tightly closed prior to freezing
- ✓ Sentinel (QC) vials must be cryopreserved using a method approved by Novartis

- ✓ Controlled-rate freeze program must be approved by Novartis
- ✓ Controlled-rate freezing of the cryobag(s) is required. Freezing by placing directly into a fixed-temperature freezer (“dump/passive freezing”) is not permitted
- ✓ Adequate space must be maintained between cryobags while freezing
- ✓ Institutional SOPs must be followed to monitor the chamber and leukapheresis material temperatures, eg, by attaching a temperature probe to one of the cryobags or sentinel (QC) vials
- ✓ The temperature of the cells must be at least −80°C before removal from the controlled-rate freezer for subsequent storage
- ✓ Following removal from the controlled-rate freezer, the cryopreserved leukapheresis material and sentinel (QC) vials must be stored to equilibrate to cryopreservation temperatures for at least one (1) hour
- ✓ ***Note:*** *Alternate freezing methods or configurations must be approved through the Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement*

*Guidance*

- **It is highly recommended to cryopreserve as soon as possible after collection, or on the same day whenever possible**
- Minimizing the time between addition of DMSO and initiation of the controlled-rate freezing cycle is critical to limit exposure of fresh cells to DMSO
- It is recommended to cryopreserve the sentinel (QC) vials along with the cryobags in the same cryopreservation procedure/run
- Only one patient's leukapheresis material should be cryopreserved in a controlled-rate freezer chamber at the same time to prevent mix-ups

*Guidance on Controlled-Rate Freezing Program*

A program that meets the following parameters is considered preapproved by Novartis and may be used with no additional approval documentation:

- Equilibrate the samples with the chamber temperature
- Cool at −1°C to −2°C per minute until reaching at least −40°C, with compensation for the eutectic point
- Once at −40°C, cool at −10°C per minute until the sample temperature probe reaches at least −80°C

**Note:** Use of an alternate program may be possible. Upon evaluation and approval, the alternate program should be documented in an approved Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement or other signed documentation from Novartis.

Table 1 on the following page provides an example of a Novartis-approved controlled-rate freezing program and may be used with no additional approval documentation.

*(Guidance continued on following page)*

*Guidance on Controlled-Rate Freezing Program (continued)*

**Table 1. Example of a Novartis-approved controlled-rate freezing program**

| Step No. | Mode | Rate (°C/min) | Chamber/ Sample | Target Temperature (°C) | Hold Time (min) | Description |
|---|---|---|---|---|---|---|
| 1[a,b] | Wait at | N/A | Chamber | 0 | 0 | Equilibration |
| 2 | Cool | 1 | Chamber | −5 | 15 | Cool −1°C/minute |
| 3 | Cool | 25 | Chamber | −47 | 1 | Eutectic compensation |
| 4 | Warm | 10 | Chamber | −9 | 0 | Eutectic compensation |
| 5 | Cool | 1 | Chamber | −40 | 0 | Cool −1°C/minute |
| 6 | Cool | 10 | Chamber | −90 | 10 | Cool −10°C/minute |
| 7[c] | END | | | | | |

N/A, not applicable; SOP, standard operating procedure. [a] Ensure that the controlled-rate freezer chamber temperature has reached 0°C, and then load the cryobag(s) and sentinel (QC) vials into the controlled-rate freezer chamber, attaching sample thermocouple probe to cryobag(s) or vial(s) according to institutional SOP. Close chamber door. [b]As program continues in Step 1, wait until sample temperature reaches ≤10°C, and then advance the program to Step 2. [c] After the end of the controlled-rate freezing program, ensure that the sample temperature has reached −80°C or colder, and then remove cryobag(s) and sentinel (QC) vials from the chamber and transfer to ≤ −120°C storage.

# Storage of Cryopreserved Cells

## *Requirements*

- ✓ Equilibrate the cryopreserved leukapheresis material in the vapor phase of liquid nitrogen ($LN_2$) (or ≤ −120°C) for at least one (1) hour after cryopreservation in the controlled-rate freezer, until packing for shipment
- ✓ Storage of cryopreserved leukapheresis material must be in a vapor phase of $LN_2$ (or ≤ −120°C) temperature-monitored system until packing for shipment
- ✓ Store cryopreserved sentinel (QC) vials at ≤ −120°C, or via a cryogenic storage method approved by Novartis, until packing for shipment
- ✓ If storing using liquid nitrogen, cells must be kept in the vapor phase of $LN_2$
- ✓ Maximum storage is 30 months
    - Cryopreserved leukapheresis material collected more than 30 months before the start of product manufacture may **NOT** be shipped to Novartis without prior approval by Novartis

# Documentation of the Cell Processing Procedure

### *Requirements*

- ✓ Steps performed and data generated must be documented in institutional records following good documentation practices and must be sufficiently detailed to provide a complete history of the process
    - For example, the volume of the leukapheresis collection must be documented in order to calculate TNC and total CD3+ for the collection, and the volume within each cryobag must be documented
- ✓ Record test results and the cryopreservation date/time on institutional forms. Results will later be entered into CellChain™

    - Cryopreservation Date/Time: The date and time at which the cryoprotectant (dimethyl sulfoxide [DMSO]) is added to the leukapheresis material
- ✓ Information pertaining to materials and reagents used in the procedure must be documented in institutional records. This information includes:
    - Manufacturer/supplier
    - Lot number
    - Expiration date

- ✓ The controlled-rate freezing curve for the cryopreserved leukapheresis material must be documented and available for Novartis to review upon request

# Routine Data and Nonroutine Event Reporting

### *Requirements*

- ✓ CellChain™ must have data entry completed and approved as soon as possible/prior to shipment of leukapheresis material
- ✓ Follow any requirements in the applicable Novartis Clinical Trial Agreement/Quality Agreement/Technical Agreement for reporting of issues that may impact the leukapheresis material to Novartis. Examples of reportable issues include:
    - Potential contamination or cross-contamination
    - Sterility failures
    - COI breaches
    - Instrument malfunctions/failures

# Best Practices for Cell Processing/Cryopreservation of Leukapheresis Material

This section provides guidance on best practices for the cell processing/cryopreservation of patient lymphocytes obtained from leukapheresis and intended for further manufacturing of cellular therapy products.

- Plan and perform a process validation for institutional cryopreservation of lymphocytes, using best practices. Include acceptance criteria for viable target cell (eg, CD3+ lymphocyte) recovery. See *Novartis Guidance for Apheresis Centers: Qualification & Validation,* available from your Novartis Cell Therapy Operations Manager
- Centrifugation for plasma or platelet removal should be done at 2°C to 8°C if possible
- To disperse cell clumping due to platelets, consider adding anticoagulant citrate dextrose solution A (ACD-A) and gently mixing. Alternatively, a low-speed centrifugation can be performed followed by removing platelet-rich supernatant. If blood filters are used, ensure the pore size allows for optimal recovery of WBCs (eg, 170-250 micron)
- If not already diluted, first dilute DMSO with electrolyte solution, allowing heat to dissipate, and then cool mixture to 2°C to 8°C
- Add protein solution (eg, HSA, autologous plasma) to chilled DMSO-electrolyte solution mixture, and then cool cryomedia mixture to 2°C to 8°C
- Pre-chill cells to 2°C to 8°C prior to adding cryomedia
- Slowly add cooled cryomedia to cells while gently mixing, at a rate consistent with the process validation and consistently from batch to batch. This will reduce osmotic stress on cells as the higher osmolality cryomedia is introduced into the physiological cell solution
- Sentinel (QC) vials should be prepared after mixing the cells with cryomedia, so that the cells cryopreserved in the vials are representative of the cells cryopreserved in cryobags
- Use cold packs or pre-cooled plates to keep cryobag contents cold when adding cryomedia and before placing into the controlled-rate freezer (CRF)
- Remove excess air bubbles from cryobags before sealing cryobag tubing. Air bubbles in cryobags can prevent effective heat removal during cooling and may increase the risk of broken bags. For each cryobag, make three complete hermetic seals close to the cryobag, and cut the middle seal

*(Best practices continued on following page)*

## **Best Practices for Cell Processing/Cryopreservation of Leukapheresis Material** (continued)

- Minimize the amount of time spent from the point when DMSO-containing cryomedia is added to the cells to the start of controlled-rate freezing. These steps should be done as quickly as possible; the total time spent in these steps should preferably be ≤20 minutes. This will reduce the biochemical toxicity of DMSO toward the cells
- Place cryobags in metal cassettes or between freezing plates, and freeze in the horizontal position within the CRF chamber to ensure an even thickness of the cellular material to allow for even cooling and consistent freezing geometrics
- It is recommended to cryopreserve the sentinel (QC) vials along with the cryobags in the same cryopreservation procedure/run
- After completion of the CRF run, transfer cryobags and sentinel (QC) vials from the CRF to a storage unit as quickly as possible. This will reduce the likelihood of partial melting of samples, which could lead to a loss of cell viability and recovery
- Cryopreserve cells on the same day as collection. Cells cryopreserved the day *after* collection generally show decreased post-thaw performance compared to cells cryopreserved on the collection day
- If cells are held overnight at 2°C to 8°C before cryopreservation, dilute the WBC concentration to ≤2 x $10^8$/mL with an electrolyte solution containing protein
- Any solutions or additives added to the cells should be brought to the same temperature as the cells prior to being added. This will reduce any temperature shock to the cells

Before making any changes to cryopreservation methods, and as with any new or changed process, an internal process validation should be performed prior to cryopreserving patient cells, as changes in timing, temperatures, equipment, containers, cryomedia, etc. can affect performance.

For more information or if you have questions, please contact Novartis Cell Therapy Operations.

References:


- Novartis Guidance for Apheresis Centers: Qualification & Validation.
- Yu G, Hubel A. The role of preservation in the variability of regenerative medicine products. *Regen Eng Transl Med*. 2019;5(4):323-331.
- Fahy GM. The relevance of cryoprotectant “toxicity” to cryobiology. *Cryobiology*, 1986;23(1):1-13.
- Hubel A. *Preservation of Cells: A Practical Manual*. John Wiley & Sons; 2018.

# Additional Notes

*Guidance on Calculating Cell Counts*

Total CD3+ lymphocyte count can be calculated using the following equation:

**Equation 1. Calculating total CD3+ lymphocyte count**

**Total CD3+ lymphocyte count** = **CD3+ lymphocyte count (cells/µL)** × **Leukapheresis material volume (mL)** × **Dilution factor[a]** × **$10^3$ µL/mL**

**(Units for each variable are given in parentheses)**

**CD3+ Lymphocyte Count**

- Obtained from flow cytometry
- Usually reported as cells per microliter

**Leukapheresis Material Volume**

- Obtained through volume calculations performed during leukapheresis material testing

**Dilution factor[a]**

- If the sample was diluted before flow cytometry, multiply here by the dilution factor used. For example, multiply by 10 if a 1:10 dilution was performed

[a]Ensure that the dilution factor is incorporated into any calculations to ensure accurate data reporting.

Total nucleated cell (TNC) count can be calculated using the following equation:

**Equation 2. Calculating TNC count**

**TNC count** = **WBC count (cells x $10^6$/mL)** × **Leukapheresis material volume (mL)** × **Dilution factor[a]**

**(Units for each variable are given in parentheses)**

**WBC count**

- Obtained from the CBC
- Usually reported as WBC x $10^6$/mL

**Leukapheresis Material Volume**

- Obtained through volume calculations performed during leukapheresis material testing

**Dilution Factor[a]**

- If the sample was diluted before running the CBC, multiply here by the dilution factor used. For example, multiply by 10 if a 1:10 dilution was performed

CBC, complete blood count; TNC, total nucleated cell; WBC, white blood cell.
[a]Ensure that the dilution factor is incorporated into any calculations to ensure accurate data reporting.

# Packing and Shipping: Cryoport Dry Vapor Shipper

## Chapter Purpose

This chapter defines the requirements and guidance for the packing and shipping of cryopreserved leukapheresis material collected for product manufacturing.

## Preparing for Shipment of Leukapheresis Material

*Background*

The cryopreserved leukapheresis material will be packed and shipped to the Novartis manufacturing facility. Shipment of cryopreserved leukapheresis material will occur using a specialty courier and must be shipped in the Novartis-provided dry vapor shipper delivered by the courier.

A dry vapor shipper is designed for the safe transportation of biological samples at cryogenic temperatures and consists of two components:

- A protective outer shipping container
- An enclosed dewar charged with liquid nitrogen. Within the dewar, the liquid nitrogen is absorbed in a foam retention system

Temperatures will be continuously monitored during shipment by a temperature probe inside the dewar connected to a data logger integrated into the lid of the dewar.

There are two (2) possible shipper configurations: the Cryoport dry vapor shipper (detailed in this section) and the evo®* DV10 dry vapor shipper (detailed in Appendix 2).

Novartis will supply your site in advance with required packing and shipping materials (Apheresis Packing Kits), as detailed in Table 1 on the following page. Contact your Novartis Customer Service Center or Novartis Cell Therapy Operations for shipment of Apheresis Packing Kits to your cell processing laboratory.

*evo* is a registered trademark of BioLife Solutions, Inc.

*Background (continued)*

**Table 1. Materials required for Cryoport leukapheresis material shipment**

| | |
|---|---|
| **Supplied by Novartis (Apheresis Packing Kits)** | Metal Cassettes[a] |
| | Inner leak-proof polybags |
| | Absorbent sheets |
| | Tyvek[b] bags |
| **Supplied by Novartis Courier (Delivered with dry vapor shipper)** | Zip ties |
| | Tamper-evident serialized zip tie |
| | Do Not X-Ray label |
| | Exempt Human Specimen label |

[a]Metal cassettes may be provided by Novartis. Institutional metal cassettes may also be used.
[b]*Tyvek* is a registered trademark of E. I. du Pont de Nemours and Company.

## *Requirements*

- ✓ The Cryoport dry vapor shipper can hold a maximum of **4 cryobags plus sentinel (QC) vials or 5 cryobags**
  - ***Note:*** *A maximum of 10 cryobags may be sent to Novartis for manufacture*

- ✓ Each dry vapor shipper may contain leukapheresis material from only 1 patient
- ✓ In some cases, more than one dry vapor shipper may be needed. All dry vapor shippers for the same patient must be shipped at the same time
  - Contact the Novartis Customer Service Center for assistance with ordering additional dry vapor shippers

- ✓ Delivery of the charged dry vapor shipper(s) to your site will be scheduled in advance of the anticipated shipping date
  - At the time of product order, an estimated date for shipment of cryopreserved cells to Novartis will be identified. The Novartis Customer Service Center should be contacted for any questions or changes related to delivery of the dry vapor shipper
- ✓ Depending on institutional labeling protocol, some sites may require hangtag labels received from Novartis to be attached to the cryobags before shipment can occur. Refer to Appendix 3 (Use of Novartis Hangtags) of this manual for further details on hangtag labeling requirements

- ✓ Packing for shipment must only be performed by personnel trained per institutional standard operating procedures (SOPs) for packing biological materials
- ✓ Institutional policy/procedures on liquid nitrogen handling must be followed when preparing the shipment

*Guidance*

If any required materials are missing, please contact the Novartis Customer Service Center.

It can be helpful to have additional backup tamper-evident serialized zip ties in the event that the Novartis courier one is damaged or lost. Backup tamper-evident serialized zip ties can be requested and provided by Novartis Cell Therapy Operations.

Dry ice may be needed to keep the cassettes and sentinel (QC) vials frozen during packaging if not being stored in the vapor phase of liquid nitrogen or placed directly into the dry vapor shipper.

## Overview of the Cryoport Dry Vapor Shipper

*Outer Shipping Container*

**Figure 1. Examples of a Cryoport dry vapor shipper**

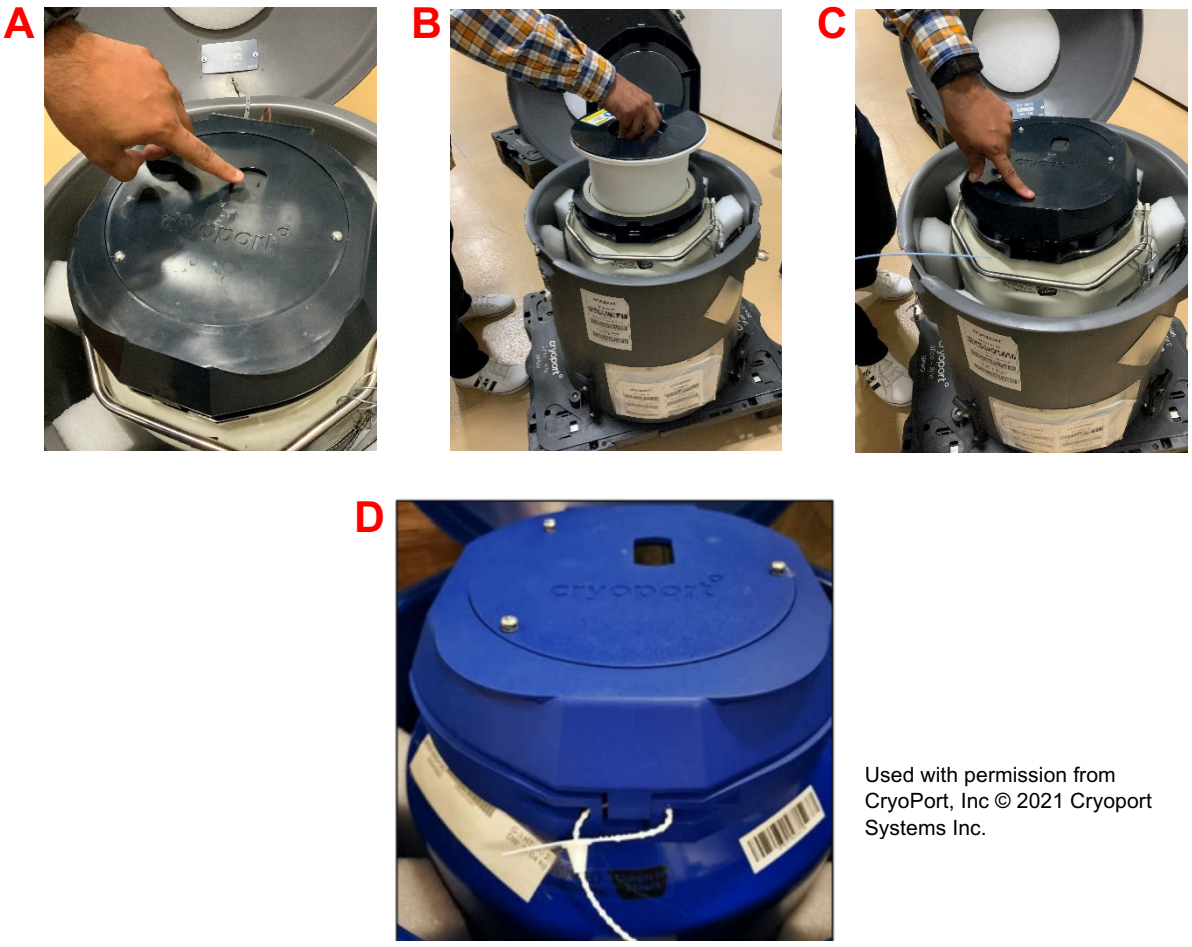

- The outer shipper plastic may vary in color (eg, gray, blue, tan)
- The outer shipping container latches may be held closed with zip ties or tamper-evident serialized zip ties; either is acceptable. It is also possible that airline security or customs may open the outer shipper and replace the original zip ties; this is acceptable

*Dewar: Updated Lid, Data Logger, and Vapor Plug*

An updated Cryoport shipper may be received. The updates include:

- A hinged lid on the dewar that contains the data logger; the lid configuration no longer includes a plastic box for the data logger (Figure 2A)
- A redesigned vapor plug with knob-style handle (Figure 2B)
- A new location for the placement of the tamper-evident serialized zip tie, which significantly improves securing the leukapheresis material and finished product during packing (Figure 2C-D)
    - Note: Close and **press down** on the hinged lid to allow for proper insertion of the tamper-evident serialized zip tie (Figure 2C-D)

**Figure 2. Updated Cryoport hinged dewar lid with data logger (A), vapor plug (B), and placement of the tamper-evident serialized zip tie (C-D)**



*Guidance on Cryoport Data Logger*

Attached to the lid of the Cryoport dewar is a protective case that houses an electronic data logger. In the updated Cryoport dewar, the data logger will be housed inside the lid. No action is needed to activate the data logger; it will continuously monitor temperature during shipping.

- Data logger functioning, tracking, and temperature recordings can be reviewed online through Cryoport's LiveView, which can be accessed through CellChain™ or via the link in the shipping notification email
- Data logger functioning may also be tested by pressing the touch-sensitive button on the front of the logger. When running your finger over this button, two green light-emitting diode (LED) indicators should appear, indicating that the data logger is working correctly. ***Note:*** *This button may be difficult to activate while wearing gloves*
- LED lights may also be lit on the data logger when the touch-sensitive button is not activated
    - A blinking green light means the logger is working
    - A blinking red light means the logger is currently transmitting information to the home base

# Receipt of the Cryoport Dry Vapor Shipper

## *Requirements*

- ✓ Verify that the batch number at the top of the incoming waybill matches the batch number for the patient in CellChain™, as each dry vapor shipper is patient specific
    - ***Note:*** *Additional information (eg, waybill number) may appear on both the incoming waybill and the Shipper Certification Form to ensure that each dry vapor shipper is assigned to the correct batch number*
- ✓ Ensure there is no damage from shipping, and verify that the dry vapor shipper is securely attached to the shipping pallet
- ✓ After opening the outer shipping container, ensure that the dewar is intact and that there is no damage from shipping
- ✓ Confirm that the pouch on the inner shipping container lid contains:
    - One tamper-evident serialized zip tie (Figure 3)
    - Zip ties
    - Do Not X-Ray label
    - Exempt Human Specimen label

**Figure 3. Example of tamper-evident serialized zip tie**




*(Requirements continued on following page)*

## *Requirements (continued)*

- ✓ Verify that the number on the tamper-evident serialized zip tie matches the number on the Shipper Certification Form (attached to shipper) (Figure 4)
  - If the number on the tamper-evident serialized zip tie does not match that on the Shipper Certification Form, or if a replacement tamper-evident serialized zip tie is to be used, the number on the Shipper Certification Form must be hand changed/corrected. Please remember to initial/sign and date any corrections to documentation to ensure Good Documentation Practices
- ✓ Verify the following numbers on the Shipper Certification Form (highlighted in Figure 4) with those on the Bar Code Label (Figure 5) on the outside of the shipper:
  - Dewar Serial Number
  - Condition Monitor Serial Number
- ✓ The Shipper Certification Form must remain with the dry vapor shipper during shipment to Novartis; copies may be made for institutional records

**Figure 4. Example of Shipper Certification Form**

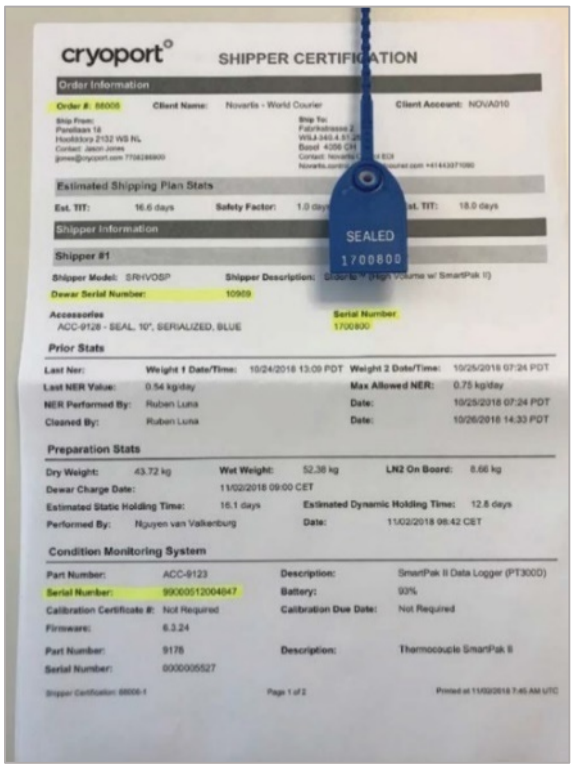



**Figure 5. Example of Bar Code Label**

# Packing the Leukapheresis Material and Sentinel (QC) Vials in the Cryoport Dry Vapor Shipper

*Background*

Specific packing requirements must be followed precisely in order to meet Novartis quality requirements and to ensure that the validated shipping requirements are met.

As described in this section, there are two available packing processes depending on the cryobags used by your institution. Both processes may use Novartis-supplied metal cassettes, or your institutional metal cassettes.

- The **Standard Cryoport Packing Procedure** is optimized for use with the Novartis-sized metal cassettes of the following dimensions: **20.1 x 13.97 x 1.11 centimeters**
- The **Oversize-Bag Cryoport Packing Procedure** is optimized for use with the Novartis-sized metal cassettes of the following dimensions: **27.1 x 15 x 1.0 centimeters**

  The Oversize-Bag Cryoport Packing Procedure should be followed if the cryobags utilized by your institution exceed the standard dimensions. Use of this procedure must be communicated to Novartis as soon as possible to ensure appropriate packing materials are supplied to your institution.

## *Requirements*

- ✓ Confirm correct labeling and patient Chain of Identity (COI) information on the cryobag(s) and sentinel (QC) vials. Novartis hangtags may be required for sites using non–International Society of Blood Transfusion (ISBT)-128 labeling. Please refer to the Leukapheresis Cell Processing chapter and Appendix 3 (Use of Novartis Hangtags) of this manual for full cryobag labeling and COI requirements
- ✓ Cryobags must be shipped in metal cassettes

- ✓ Ensure that steps are taken to prevent thawing of cryobags and sentinel (QC) vials, such as storing bagged cassettes and sentinel (QC) vials in the vapor phase of liquid nitrogen or on dry ice, if not immediately being loaded into the dry vapor shipper
- ✓ At all times, minimize the amount of time the dewar lid is open

*(Requirements continued on following page)*

## *Requirements (continued)*

*Standard Packing and Dewar Loading*

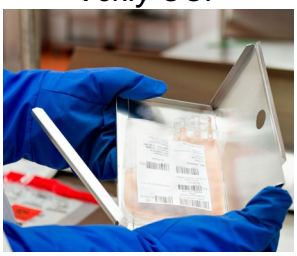
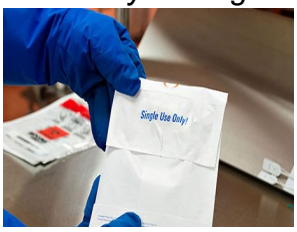
- ✓ Each metal cassette must be packed with at least 1 absorbent sheet and sealed in a leak-proof polybag
- ✓ Sentinel (QC) vials must be packed with at least 1 absorbent sheet and sealed in a leak-proof polybag
- ✓ Each sealed polybag must be packed and sealed in a Tyvek outer bag
- ✓ Load a maximum of four (4) bagged (polybag + Tyvek bag) cryobags/cassettes plus bagged sentinel (QC) vials, or five (5) bagged cryobags/cassettes, into the holder/metal rack in a single dewar/dry vapor shipper

*Oversize-Bag Packing and Dewar Loading*

- ✓ Each metal cassette must be packed with at least 4 absorbent sheets and sealed in a leak-proof polybag
- ✓ Sentinel (QC) vials must be packed with at least 1 absorbent sheet and sealed in a leak-proof polybag
- ✓ Load a maximum of four (4) bagged (polybag) cryobags/cassettes plus sentinel (QC) vials, or five (5) bagged cryobags/cassettes, into the holder/metal rack in a single dewar/dry vapor shipper
- ✓ The entire holder/metal rack must be inserted and sealed in the large Tyvek bag

*Standard Cryoport Packing Procedure*

**Figure 6. Example of Standard Cryoport Packing Procedure**

| | | |
|---|---|---|
| *Verify COI*  | *Insert cassette into polybag*  | *Add 1 or more absorbent sheets*  |
| *Seal polybag*  | *Fold polybag*  | *Insert into Tyvek bag*  |
| *Seal Tyvek bag*  | *Fold Tyvek bag*  | *Insert into metal rack*  |

COI, Chain of Identity.

**Notes:**

- It is important to ensure that metal cassettes are precooled before inserting a cryopreserved bag in order to prevent micro-thaws, which can damage cells
- Packing cassettes port side up is strongly advised, as this may help to prevent possible damage during shipment
- When packing and wrapping with the polybag and the Tyvek bag, follow any manufacturer-supplied directions
- It may be a tight fit to load the bagged cassettes containing the cryopreserved leukapheresis material and the bagged sentinel (QC) vials into the holder/metal rack. Ensuring a tight wrapping with the polybag and Tyvek bag, and expelling excess air prior to sealing bags, can help make insertion into the rack easier
- When opening the dewar lid, take care not to dislodge any wires from the temperature monitor that may be attached to the dewar lid

*Oversize-Bag Cryoport Packing Procedure*

**Figure 7. Example of Oversize-Bag Cryoport Packing Procedure**

*Verify COI*

*Insert cassette into polybag*

*Add 4 or more absorbent sheets*

*Seal polybag*

*Fold polybag*

*Insert into metal rack*

*Seal metal rack into large Tyvek bag*

**Notes:**

- It is important to ensure that metal cassettes are precooled before inserting a cryopreserved bag in order to prevent micro-thaws, which can damage cells
- It is strongly advised to keep track of the port orientation on bags during the packing process and to ensure that bags are loaded into the dewar port side up, as this may help to prevent possible damage during shipment
- When packing and wrapping with the polybag and the Tyvek bag, follow any manufacturer-supplied directions

*(Continued on following page)*

*Oversize-Bag Cryoport Packing Procedure (continued)*

- Try to expel as much air as possible from the polybag before sealing. Expelling excess air can make insertion into the dewar rack easier
- When opening the dewar lid, take care not to dislodge any wires from the temperature monitor that may be attached to the dewar lid
- The handles on the large Tyvek bag containing the packed leukapheresis material can be used to help place it inside the dewar

## Closing and Sealing the Cryoport Dry Vapor Shipper

### *Requirements*

- ✓ The time the leukapheresis material is loaded into the dewar must be documented on institutional forms. This information will need to be entered into CellChain™
- ✓ The inner dewar lid must be sealed with a tamper-evident serialized zip tie
- ✓ The outer shipping container latches must be sealed with zip ties (nonserialized zip tie(s) provided by the courier may be used)

*Closing and Sealing the Cryoport Dewar*

- ***For the current-generation dewar***, it is important to carefully align the holes on the back of the dewar lid and the holes on the neck of the dewar to ensure that the tamper-evident serialized zip tie can pass through the holes in the dewar lid and neck in order to properly seal the dewar lid (Figure 8)

**Figure 8. Example of sealed dewar (current generation)**

Used with permission from CryoPort, Inc © 2019 Cryoport Systems Inc.

*(Continued on following page)*

*Closing and Sealing the Cryoport Dewar (continued)*

- ***For the updated dewar,*** it is important to first close and **press down** on the hinged lid of the dewar (Figure 9A). This motion will align the holes on the dewar lid and neck of the dewar. Thread the tamper-evident serialized zip tie through the holes in the dewar lid and neck, and then seal (Figure 9B). Ensure that the dewar has been properly sealed and cannot be opened

**Figure 9. Example of sealing the dewar (updated generation): pressing down on closed dewar lid (A) and sealing with tamper-evident serialized zip tie (B)**

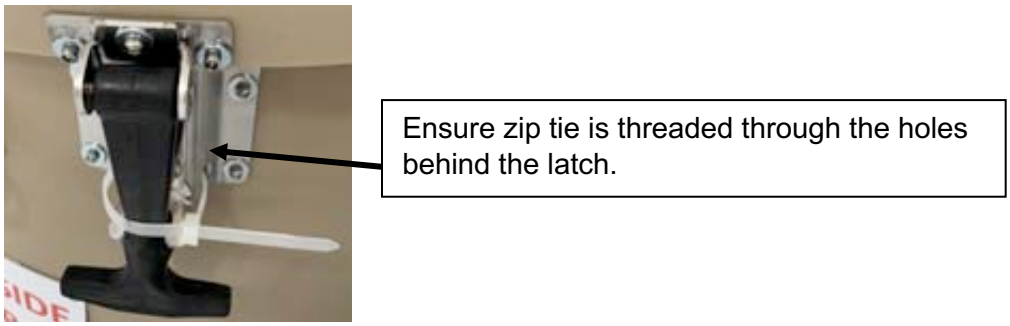
A

B


- ***Note:*** If the tamper-evident serialized zip tie provided by the courier is lost or damaged, a backup or institutional tamper-evident serialized zip tie may be used. If a backup tamper-evident serialized zip tie is used, ensure that the Shipper Certification Form is updated utilizing Good Documentation Practices to strike through the original number and write in the new serial number as it appears on the zip tie. Initial and date the change

*Closing and Sealing the Cryoport Outer Shipping Container*

✓ Seal the latches with the zip ties



# Shipment Documentation and Labeling

## *Requirements*

- ✓ Ensure that the “Do Not X-Ray” label and “Exempt Human Specimen” label are properly adhered to the outer shipping container
- ✓ Four copies of the signed outgoing waybill must be placed in the pouch on the outside of the dry vapor shipper (in some regions the courier may provide the copies of the outgoing waybill). One copy will remain with the dry vapor shipper at all times. The other three copies are one for the courier driver, one for the airport, and one for Novartis records
- ✓ The Shipper Certification Form must remain with the shipper; copies may be made for institutional records
- ✓ Ensure that documentation in CellChain™ is complete and verified prior to the shipment
- ✓ The time the loaded dry vapor shipper is released to the courier must be documented on institutional forms. This information will need to be entered into CellChain™

# Appendix 1: Leukapheresis in Small Patients

## Appendix Purpose

This appendix is intended to list Novartis' recommendations for physicians on how to perform a safe and efficient leukapheresis to obtain starting material for patients weighing 25 kg or less (ie, "small-sized" patients).

## Recommendations

*Patient Readiness*

Confirm the patient has been off medications per the recommended washout periods prior to apheresis. Please contact Novartis Medical if you should have questions.

Patients with high circulating peripheral lymphoblasts may have challenges with collection and meeting the requirements for the CD3+ cell count and CD3+% of the leukapheresis material. Leukapheresis material with high-blast content may likely affect product manufacturing success. Therefore, treating physicians should attempt therapy to reduce the number of circulating lymphoblasts prior to leukapheresis in patients with pediatric acute lymphocytic leukemia (ALL) with very high circulating lymphoblast counts.

*Venous Access*

It is recommended to use Central Venous Catheters (CVC) when performing leukapheresis collections with patients weighing less than 25 kg. Work with the surgeons or interventional radiology to choose the most appropriate central venous catheter that will not collapse during the collection.

*Blood Prime*

Blood prime is recommended for small patients who weigh less than 25 kg since the extracorporeal volume is >10-15% of their total blood volume (TBV). The newer apheresis instruments have a warning to consider using a custom blood prime.

### *Hypothermia*

It is recommended to use a blood warmer in line if available or warmed blankets to prevent the patient from getting chills/hypothermia.

### *Anticoagulant and Electrolytes*

Anticoagulant citrate dextrose solution A (ACD-A) alone may cause hypocalcemia in low-weight children. Physicians may choose to use an ACD-A and heparin mix when collecting from patients weighing less than 25 kg. This may allow an increased inlet rate since the patient would be receiving less ACD-A. Monitoring patients for signs/symptoms of hypocalcemia, hypomagnesemia, and alkalosis can be challenging with patients who cannot tell you they are having symptoms such as circumoral tingling; they may tend to cry and be inconsolable. Monitoring the patient's electrolytes is necessary (eg, ionized Ca, Mg) in addition to monitoring vital signs (heart rate, blood pressure, etc).

### *Collection*

This is a **non-mobilized mononuclear cells (MNC)** collection. Monitor the collect line color and rate. Most instruments will default to 0.8 to 1.2 mL/min collect rate or slower for smaller patients. If you are collecting based on interface color, try decreasing the collect rate to optimize the collection. The color goal of the collect line should be a light salmon color.

Monitor the leukapheresis volume collected. Per the American Association of Blood Banks (AABB) standards, no more than 10 mL/kg of blood should be outside of a patient's body at any given time during the collection.

### *How Much to Process/Multiple Collections*

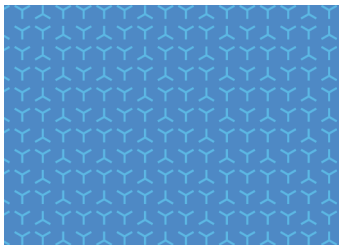
It is recommended to confirm the patient's CD3+ and absolute lymphocyte count (ALC) at least the day before the collection. Processing 2 to 3x the patient's TBV in one day may be a challenge for the patient, as this may take several hours. Small patients may require a second day of collection if their peripheral blood CD3+ count is lower than 100 cells/µL. It is recommended to discuss the potential need for a second collection with the family so they are aware and know what to expect.

*Partial Rinseback*

Utilizing partial rinseback or reinfusing the red blood cells at the end of the collection rather than saline is an option to increase the patient's Hct at the end of the leukapheresis collection. Using a partial rinseback may prevent the need for a transfusion after the leukapheresis collection.

References:


- Kim HC. Therapeutic pediatric apheresis. *J Clin Apher.* 2000;15(1-2):129-157.
- Sörensen J, Jarisch A, Smorta C, et al. Pediatric apheresis with a novel apheresis device with electronic interface control. *Transfusion*. 2013;53(4):761-765.
- Michon B, Moghrabi A, Winikoff R, et al. Complications of apheresis in children. *Transfusion.* 2007;47(10):1837-1842.



# Appendix 2: Packing and Shipping: evo® DV10 Dry Vapor Shipper

## Appendix Purpose

This appendix defines the requirements for the packing and shipping of cryopreserved leukapheresis material collected for product manufacturing using the evo® DV10 dry vapor shipper and also provides supplemental guidance and examples related to Novartis' packing configuration for the evo® DV10.

## Preparing for Shipment of Leukapheresis Material

*Background*

The cryopreserved leukapheresis material will be packed and shipped to the Novartis manufacturing facility. Shipment of cryopreserved leukapheresis material will occur using a specialty courier and must be shipped in the Novartis-provided dry vapor shipper delivered by the courier.

A dry vapor shipper is designed for the safe transportation of biological samples at cryogenic temperatures and consists of two components:

- A protective outer shipping container
- An enclosed dewar charged with liquid nitrogen. Within the dewar, the liquid nitrogen is absorbed in a foam retention system

Temperatures will be continuously monitored during shipment by a temperature probe inside the dewar connected to a data logger integrated into the lid of the dewar.

There are two (2) possible shipper configurations: the Cryoport dry vapor shipper (instructions detailed in the Packing and Shipping chapter) and the evo® DV10 dry vapor shipper (instructions detailed in this appendix).

Novartis will supply your site in advance with required packing and shipping materials (Apheresis Packing Kits), as detailed in Table 1 on the following page. Contact your Novartis Customer Service Center or Novartis Cell Therapy Operations for shipment of Apheresis Packing Kits to your cell processing laboratory.

*Background (continued)*

**Table 1. Materials required for evo® DV10 leukapheresis material shipment**

| | |
|---|---|
| **Supplied by Novartis (Apheresis Packing Kits)** | Metal Cassettes[a] |
| | Inner leak-proof polybags |
| **Supplied by Novartis Courier (Delivered with dry vapor shipper )** | Tamper-evident serialized zip tie |
| | Zip tie |
| | Exempt Human Specimen label |
| | Do Not X-Ray label |
| | ModPak containing Tyvek bag and absorbent pads |

[a]Metal cassettes may be provided by Novartis. Institutional metal cassettes may also be used.

## *Requirements*

- ✓ The evo® DV10 dry vapor shipper can hold a maximum of **4 cryobags plus sentinel (QC) vials**
  - ***Note:*** *A maximum of 10 cryobags may be sent to Novartis for manufacture*
- ✓ Each dry vapor shipper may contain leukapheresis material from only 1 patient

- ✓ In some cases, more than one dry vapor shipper may be needed. All dry vapor shippers for the same patient must be shipped at the same time
  - Contact the Novartis Customer Service Center for assistance with ordering additional dry vapor shippers

- ✓ Delivery of the charged dry vapor shipper(s) to your site will be scheduled in advance of the anticipated shipping date
  - At the time of product order, an estimated date for shipment of cryopreserved cells to Novartis will be identified. The Novartis Customer Service Center should be contacted for any questions or changes related to delivery of the dry vapor shipper
- ✓ Depending on institutional labeling protocol, some sites may require hangtag labels received from Novartis to be attached to the cryobags before shipment can occur. Refer to Appendix 3 (Use of Novartis Hangtags) of this manual for further details on hangtag labeling requirements

- ✓ Packing for shipment must only be performed by personnel trained per institutional standard operating procedures (SOPs) for packing biological materials
- ✓ Institutional policy/procedures on liquid nitrogen handling must be followed when preparing the shipment

*Guidance*

If any required materials are missing, please contact the Novartis Customer Service Center.

It can be helpful to have additional backup tamper-evident serialized zip ties in the event that the Novartis courier one is damaged or lost. Backup tamper-evident serialized zip ties can be requested and provided by Novartis Cell Therapy Operations.

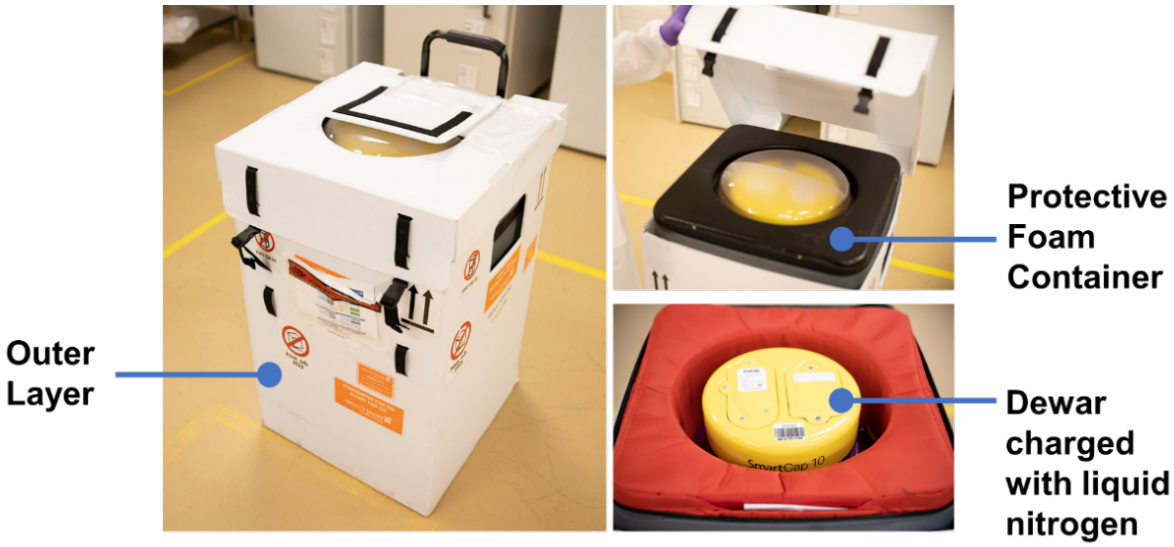
Dry ice may be needed to keep the cassettes and sentinel (QC) vials frozen during packaging if not being stored in the vapor phase of liquid nitrogen or placed directly into the dry vapor shipper.

## Overview of the evo® DV10 Dry Vapor Shipper

*Outer Layer, Protective Foam Container, and Dewar*

The evo® DV10 dry vapor shipper consists of an outer layer surrounding a protective foam container and inner dewar charged with liquid nitrogen (Figure 1).

**Figure 1. Example of an evo® DV10 dry vapor shipper**




*(Continued on following page)*

*Outer Layer, Protective Foam Container, and Dewar (continued)*

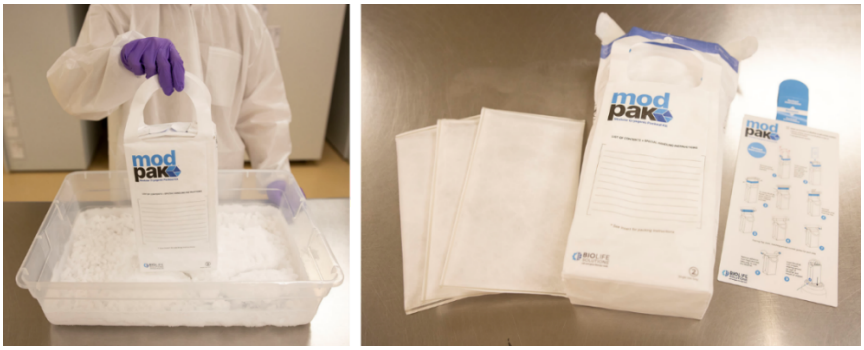
- The evo® DV10 dry vapor shipper has an integrated retractable handle and wheels for transportation
- The outer layer will include affixed pouches containing the shipping documents (eg, waybill, evoIS DV10 Certified Shipment Report)
- The colors of the outer layer and protective foam container may vary (eg, red, green, purple). This is expected, and there is no difference in the dewar contained within the outer layer and protective foam container regardless of the color
- The protective foam container may be held closed with zip ties or tamper-evident serialized zip ties; either is acceptable. It is also possible that airline security or customs may open the protective foam container and replace the original zip ties; this is acceptable
- The following labels may also be found on the evo® DV10 dry vapor shipper outer layer. If present, do not remove these labels. These may include:
    - "Do not freeze" label
    - "Keep upright" label
    - "Not stackable" label

*The DV10 ModPak*

The ModPak consists of a Tyvek overwrap bag that contains absorbent pads (Figure 2). The Tyvek overwrap acts as a rack that holds the bagged leukapheresis material and sentinel (QC) vials. The ModPak includes additional instructions on how to properly seal the ModPak using the integrated adhesive strips.

The ModPak required for shipment will be delivered inside the dewar of the evo® DV10 dry vapor shipper. Please contact the Novartis Customer Service Center if there is no ModPak in the dewar upon receipt.

**Figure 2. Example of the ModPak**

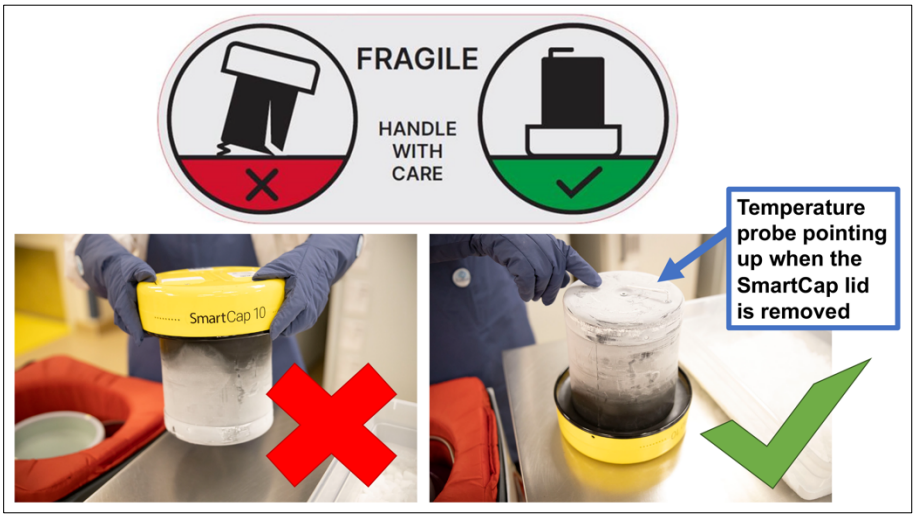


*The DV10 SmartCap With Integrated Data Logger*

The dewar lid (SmartCap) has a built-in data logger and a temperature probe integrated into the bottom of the lid. **No action is needed to activate the data logger**; it will continuously monitor temperature during shipping.

- When removed from the dewar, **the SmartCap should be placed on a flat surface upside down with the temperature probe pointing up to prevent damage of the probe** (Figure 3)

**Figure 3. Example of the SmartCap and incorrect (left) and correct (right) positioning during retrieval or loading of the ModPak**




*(Continued on following page)*

*The DV10 SmartCap With Integrated Data Logger (continued)*

- Exposure of the temperature probe to ambient temperature outside of the dewar may lead to a recorded temperature excursion (dewar internal temperature > −120°C). As such, temperature excursions may be recorded during retrieval or loading of the ModPak and are acceptable
- The SmartCap may have a temperature button that can be pressed to verify the dewar inner temperature (Figure 4). This temperature button is not relevant for the use of the evo® DV10 dry vapor shipper
    - The light will remain constant if the dewar temperature is within range (dewar internal temperature < −120°C)
    - The light will blink if the Dewar temperature is outside the acceptable range (dewar internal temperature > −120°C)

**Figure 4. Example of the SmartCap temperature button (red arrow)**

## Overview of evoIS QuickLook

*evoIS QuickLook Opening Page*

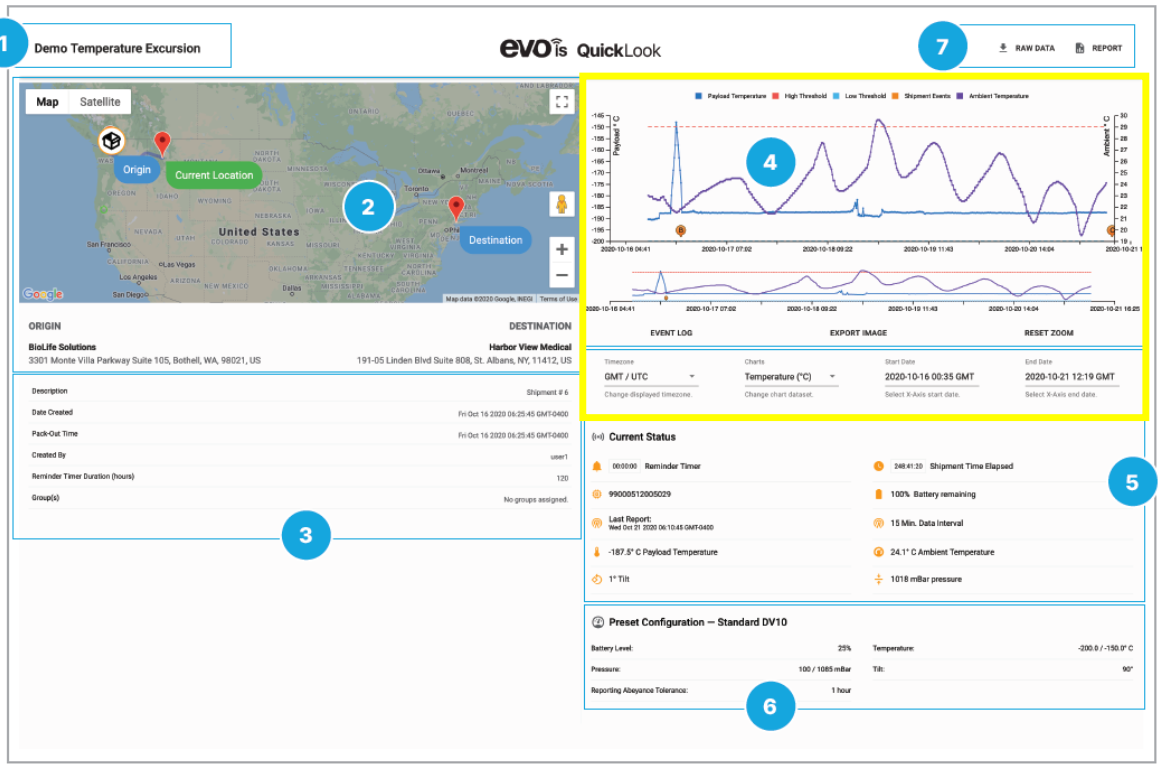
Data logger functioning, tracking, and temperature recordings can be reviewed online via the evoIS QuickLook application, which can be accessed through a link in the shipping notification email or CellChain™

- The link opens a browser that displays the shipment without the need for a username or password (Figure 5 on the following page)

*(Continued on following page)*

*evoIS QuickLook Opening Page (continued)*

**Figure 5. Example of the evoIS QuickLook opening page**




1. Origin and destination names
2. Map showing origin, destination and shipper location – addresses are shown below the map
3. Shipment overview
4. Temperature graph: provides a visual display of temperature, pressure, tilt, alerts, and critical data over the shipment journey
5. Current status of shipment: provides a summary of the evo® DV10 dry vapor shipper used, including the current status of critical metrics such as dewar internal temperature, percentage of battery remaining, tilt, and pressure
6. Shipper preset configuration: provides an overview of the preset thresholds configured for the evo® DV10 dry vapor shipper
7. Raw data and report download: allows a user to download a CSV file of all data, or download a PDF copy of the Shipment Report or evoIS DV10 Certified Shipment Report

*DV10 Temperature Graph*

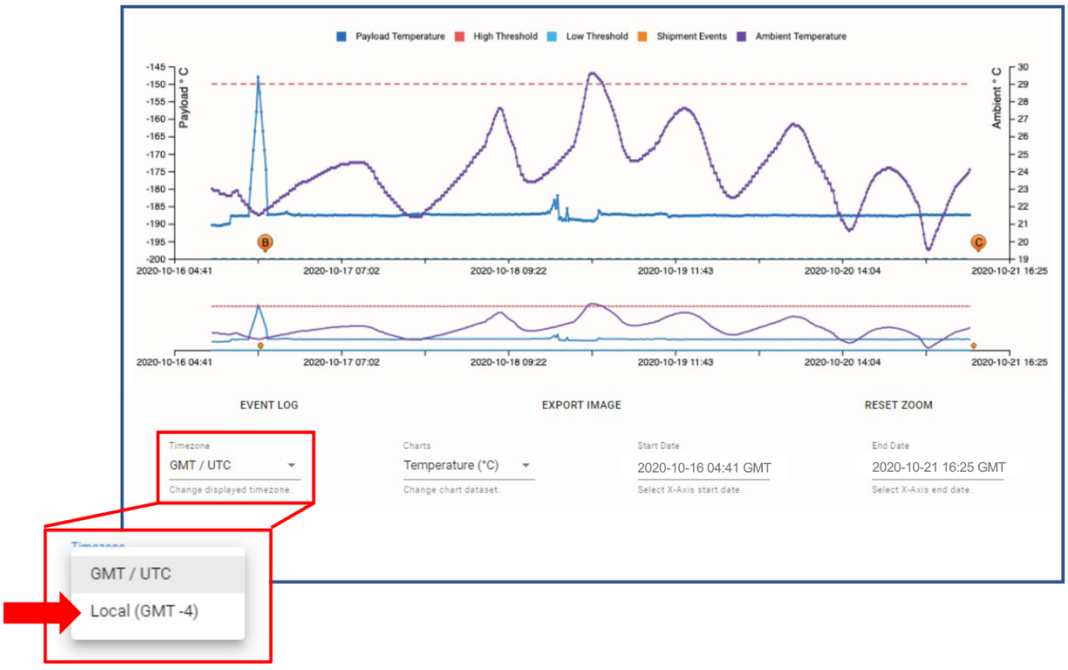
On the temperature graph within evoIS QuickLook (Figure 6), the y-axis shows temperature in °C (left scale shows internal dewar temperature, right scale shows ambient temperature). The x-axis shows the date and timeline.

The color legend in the graph indicates the line color assignment and the color of specific temperatures. In this display example:

- The dewar internal temperature is in blue, with temperature threshold limits defined by dotted lines in red (high) and blue (low)
- The ambient temperature is in purple
- Alerts are shown with an orange bubble/circle within the timeline and may indicate a temperature excursion event. Hover over the bubble to see the type of alert

**Note:** The timeline in the temperature graph may initially be displayed in the Coordinated Universal Time (UTC) time zone. To view the timeline in your local time, update the time zone in the drop-down menu below the temperature graph (Figure 6; red arrow)

**Figure 6. Example of temperature graph**



*Reports and Data*

The evoIS QuickLook application provides the capability to download the raw data from the shipment and/or a PDF copy of the Shipper Report or evoIS DV10 Certified Shipment Report.

- To download the raw data, click RAW DATA, and a CSV file will be automatically generated
- To download the Shipper Report for your records or a copy of the evoIS DV10 Certified Shipment Report, click REPORT. You can download, preview, or print the Shipper Report PDF and evoIS DV10 Certified Shipment Report (Figure 7)

**Note:** Reports exported from evoIS QuickLook will be in the Coordinated Universal Time (UTC) time zone, which can differ from your local time zone.

**Figure 7. Downloading a copy of the Shipment Report or evoIS DV10 Certified Shipment Report**

# Receipt of the evo® DV10 Dry Vapor Shipper

## *Requirements*

- ✓ Keep the evo® DV10 dry vapor shipper in its upright position when not tilted for transport
- ✓ Verify that the batch number on the top of the incoming waybill matches the batch number for the patient in CellChain™, as each dry vapor shipper is patient specific
    - ***Note:*** *Additional information (eg, waybill number) may appear on both the incoming waybill and the evoIS DV10 Certified Shipment Report to ensure that each dry vapor shipper is assigned to the correct batch number*
- ✓ Ensure there is no damage from shipping; contact the Novartis Customer Service Center if there is damage or if the shipper's wheels or handles are not working correctly
- ✓ Open the evo® DV10 dry vapor shipper to access the dewar. Ensure that the dewar is intact and that there is no damage from shipping
- ✓ Confirm that the pouch in the foam container contains (Figure 8):
    - One tamper-evident serialized zip tie
    - Zip tie
    - One "Do Not X-Ray" label
    - One "Exempt Human Specimen" label

**Figure 8. Items located in the pouch of the protective foam container**


*(Requirements continued on following page)*

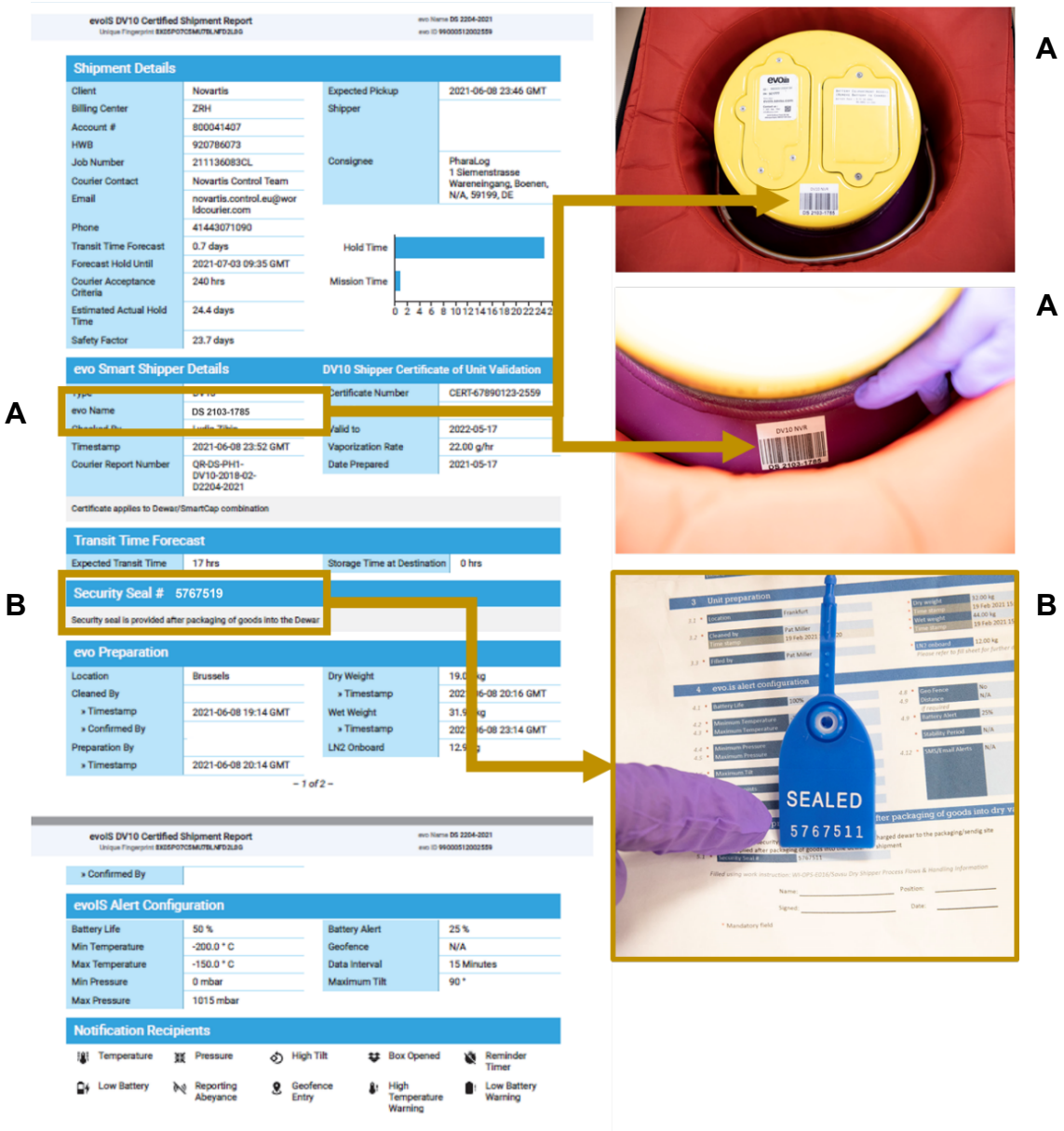
***Requirements** (continued)*

- ✓ Verify that the serial number on the SmartCap and the dewar matches the number on the evoIS DV10 Certified Shipment Report (provided with the shipper) (Figure 9A on the following page). The serial number on the SmartCap and the Dewar must be identical
    - When receiving multiple DV10 shippers, each SmartCap must remain with its originally assigned dewar
- ✓ Verify that the number on the tamper-evident serialized zip tie matches the number on the evoIS DV10 Certified Shipment Report (Figure 9B on the following page)
    - ***Note:** If the number on the tamper-evident serialized zip tie does not match that on the evoIS DV10 Certified Shipment Report, or if a replacement tamper-evident serialized zip tie is to be used, the number on the evoIS DV10 Certified Shipment Report must be hand changed/corrected. Please remember to initial/sign and date any corrections to documentation to ensure Good Documentation Practices*
- ✓ The evoIS DV10 Certified Shipment Report must remain with the dry vapor shipper during shipment to Novartis; copies may be made for institutional records
    - ***Note:** Refer to the preceding "Overview of evoIS QuickLook" section for details on downloading an electronic copy of the evoIS DV10 Certified Shipment Report*

*(Requirements continued on the following page)*

***Requirements*** *(continued)*

**Figure 9. Examples of verification of the SmartCap and dewar serial numbers (A) and tamper-evident serialized zip tie (B) against the evoIS DV10 Certified Shipment Report**



# Packing the Leukapheresis Material and Sentinel (QC) Vials in the ModPak

*Background*

Specific packing requirements must be followed precisely in order to meet Novartis quality requirements and to ensure that the validated shipping requirements are met. The leukapheresis material and sentinel (QC) vials will be packed into a ModPak.

The packing processes for the evo® DV10 dry vapor shipper may use either the standard or larger-sized metal cassettes provided by Novartis, or your institutional metal cassettes of similar dimensions.

- Novartis-provided standard-sized metal cassettes of the following dimensions: **20.1 x 13.97 x 1.11 centimeters**
- Novartis-provided larger-sized metal cassettes of the following dimensions: **27.1 x 15 x 1.0 centimeters**

Please notify the Novartis Customer Service Center if you would like to be supplied with the larger-sized metal cassettes.

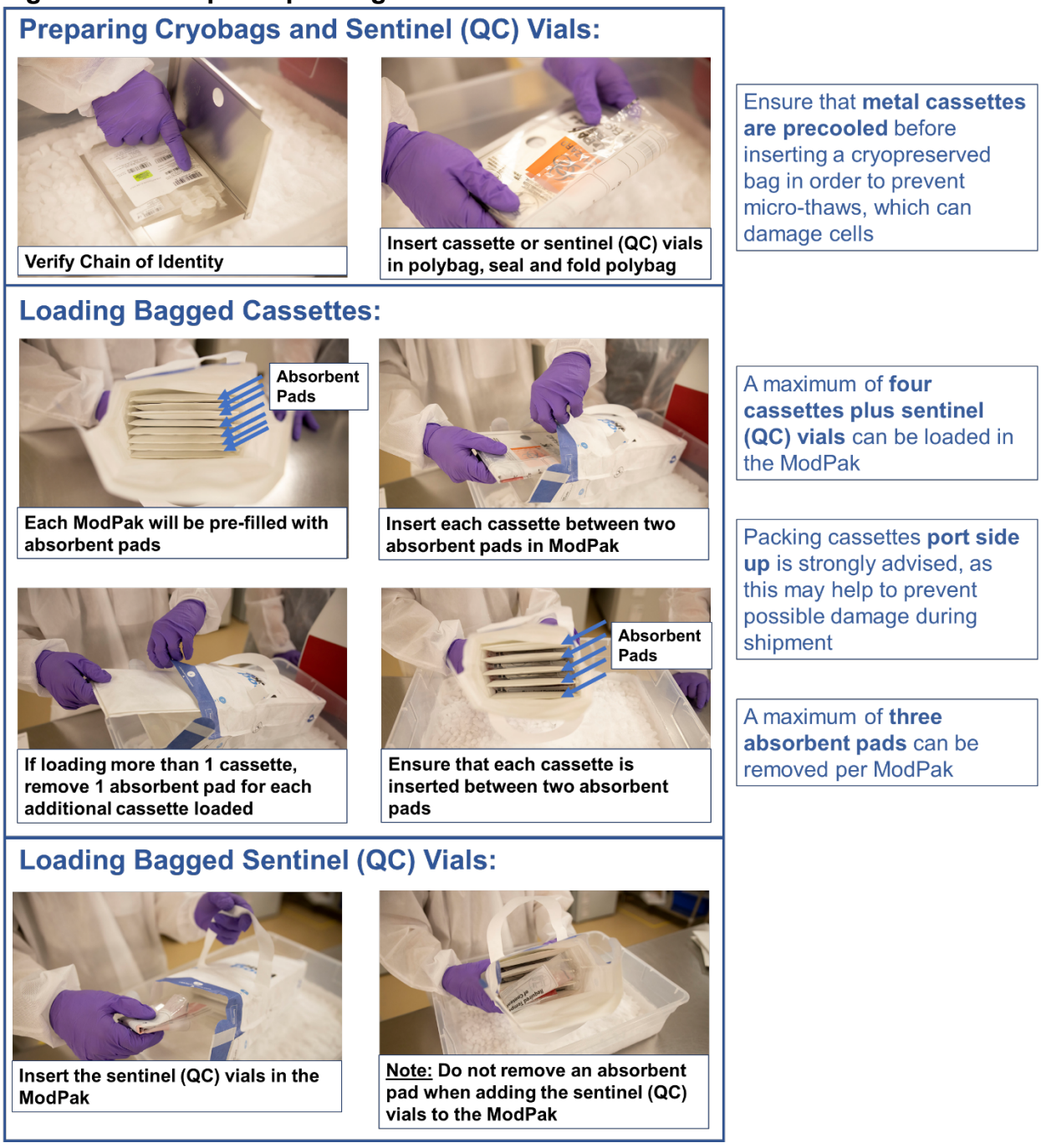
### *Requirements*

- ✓ Confirm correct labeling and patient Chain of Identity (COI) information on the cryobag(s) and sentinel (QC) vials. Novartis hangtags may be required for sites using non–International Society of Blood Transfusion (ISBT)-128 labeling. Please see the Leukapheresis Cell Processing chapter and Appendix 3 (Use of Novartis Hangtags) for full cryobag labeling and COI requirements

- ✓ Ensure that steps are taken to prevent thawing of cryobags and sentinel (QC) vials, such as storing bagged cassettes and sentinel (QC) vials in the vapor phase of liquid nitrogen or on dry ice, if not immediately being loaded into the dry vapor shipper
- ✓ Cryobags must be shipped in metal cassettes
- ✓ Each cryobag/cassette must be sealed in a leak-proof polybag
- ✓ Sentinel (QC) vials must be sealed in a leak-proof polybag
- ✓ A maximum of four cryobags/cassettes plus sentinel (QC) vials can be loaded into the ModPak
- ✓ Ensure that each cassette is inserted between two absorbent pads
    - If loading more than one cassette into the ModPak, it is recommended to remove one absorbent pad for each additional cassette loaded
    - A maximum of three absorbent pads can be removed
- ✓ Sentinel (QC) vials must be packed into the ModPak, in one slot between two absorbent pads or on the top of the cassettes within the ModPak
    - ***Note:*** *Do not remove an absorbent pad when inserting the sentinel vials*
- ✓ The ModPak must be sealed
    - Once a ModPak is closed, it should not be reopened. Please contact Novartis Cell Therapy Operations if the ModPak had to be reopened during packaging

*evo® DV10 Packing Procedure*

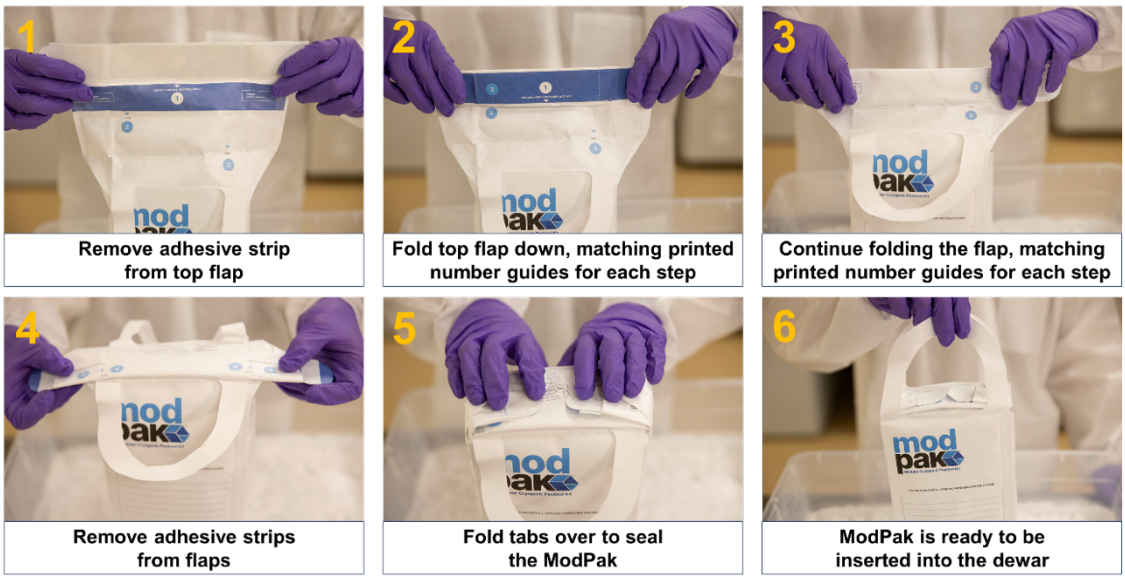
**Figure 10. Example of packing the ModPak**




*(Continued on following page)*

*evo® DV10 Packing Procedure (continued)*

**Figure 11. Example of sealing the ModPak**




***Note:*** *Additional instructions for sealing the ModPak using its dedicated adhesive strips are provided inside each ModPak.*

## Loading the ModPak into the Dewar, and Closing and Sealing the Dry Vapor Shipper

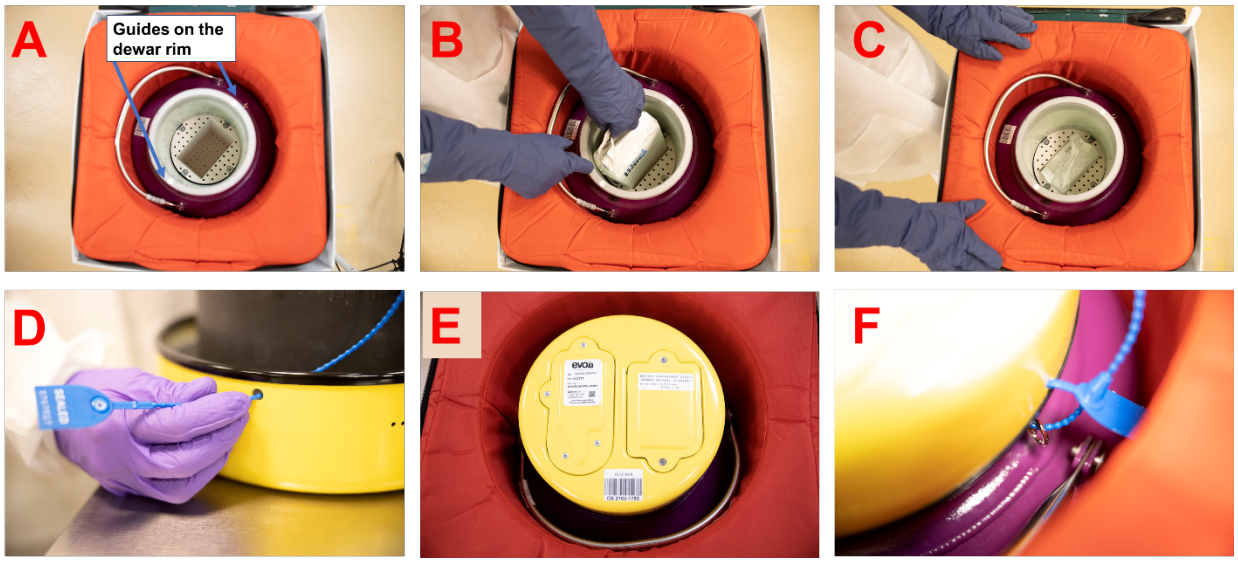
### *Requirements*

- ✓ The entire sealed ModPak must be loaded into the fitted slot in the dewar
  - ***Note:*** *Attention is needed to avoid damaging the integrated temperature monitor probe on the SmartCap when opening the dewar*
- ✓ At all times, minimize the amount of time the dewar lid is open
- ✓ The time the leukapheresis material is loaded into the dewar must be documented on institutional forms. This information will need to be entered into CellChain™
  - ***Note:*** *The time entered into CellChain™ will be used by Novartis to assess any temperature spikes recorded by the data logger during packing of the leukapheresis material*
- ✓ The dewar must be firmly closed using the SmartCap and sealed with a tamper-evident serialized zip tie
- ✓ Close the lid of the protective foam container, close the zippers, and seal with the provided zip tie
- ✓ Close the lid of the outer layer and fasten both of the outer latches

*Loading the Sealed ModPak in the Dewar and Sealing the Dewar*

- Guides on the dewar rim will help indicate the orientation of the ModPak to fit it into its fitted slot (Figure 12A-B)
- Lower the ModPak into its fitted slot, and press down firmly to secure the ModPak in the bottom of the fitted slot (Figure 12C)
- Thread the tamper-evident serialized zip tie through the hole of the SmartCap (Figure 12D) before placing the SmartCap on the dewar
    - If the tamper-evident zip tie provided by the courier is lost or damaged, a backup or institutional tamper-evident zip tie may be used. If a backup tamper-evident zip tie is used, ensure that the evoIS DV10 Certified Shipment Report is updated utilizing Good Documentation Practices to strike through the original number and write in the new serial number as it appears on the zip tie. Initial and date the change
- It may be necessary to push down firmly with both hands to make sure that the SmartCap is fully seated in the dewar (Figure 12E)
- Line up the SmartCap hole with the eyelet on the neck of the dewar
- Thread the tamper-evident serialized zip tie on the SmartCap through the eyelet, thereby binding the SmartCap and dewar together (Figure 12F)
- Seal the tamper-evident serialized zip tie (Figure 12F)

**Figure 12. Example of ModPak placement in the dewar and dewar sealing**



*Closing and Sealing the Protective Foam Container and Outer Layer*

- Close the lid of the protective foam container with the provided zip tie (Figure 13 A-B)
- Close the lid of the outer layer and fasten both of the outer latches (Figure 13C)

**Figure 13. Example of sealing the protective foam container and outer layer**


## Shipment Documentation and Labeling

### *Requirements*

- ✓ Ensure the "Do Not X-Ray" label and "Exempt Human Specimen" label are properly adhered to the outer layer (Figure 13C, above)
- ✓ Four copies of the signed outgoing waybill must be placed in the pouch on the outside of the dry vapor shipper (in some regions the courier may provide the copies of the outgoing waybill). One copy will remain with the dry vapor shipper at all times. The other three copies are one for the courier driver, one for the airport, and one for Novartis records
- ✓ The evoIS DV10 Certified Shipment Report must remain with the shipper; copies may be made for institutional records
    - ***Note:*** *An electronic copy of the evoIS DV10 Certified Shipment Report can be obtained from evoIS QuickLook, accessible via CellChain™. (Refer to the "Overview of evoIS QuickLook" section of this chapter for further details.)*
- ✓ Ensure that documentation in CellChain™ is complete and verified prior to the shipment
- ✓ The time the loaded dry vapor shipper is released to the courier must be documented on institutional forms. This information will need to be entered into CellChain™

# Appendix 3: Use of Novartis Hangtags

## Appendix Purpose

This Appendix details the use of Novartis hangtags for applicable sites.

## Cryobag Labeling Using Novartis Hangtags

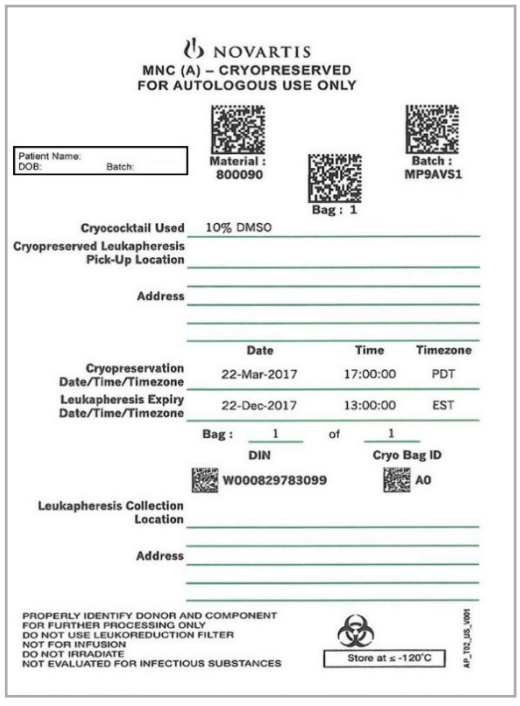
Sites may be required to use Novartis-provided hangtags (Figure 1), in addition to institutional affixed labels, if International Society of Blood Transfusion (ISBT)-128 labeling is not fully implemented (content and format) and/or to meet local country or regulatory regulations. It may be possible for Novartis to review an institutional non–ISBT-128 label to determine if the requirement to use a Novartis hangtag label may be waived.

### *Requirements*

- ✓ Hangtag labels must be attached to each cryobag before shipment to Novartis
- ✓ Hangtag labels will be printed by Novartis and shipped to the cell processing laboratory via courier
- ✓ Data entry into CellChain™ should be completed as soon as possible after cryopreservation since the hangtags contain cryobag identification information and labels cannot be generated and shipped without this information

- ✓ Verification of labels must take place by 1 qualified staff member using a validated process (eg, bar code reader) or by 2 qualified staff members

**Figure 1. Example of Novartis hangtag**



*Guidance for attaching the Novartis Hangtag Label*

Perform steps 1 and 2 prior to removing the cryobags from the freezer:

1. Verify accurate printing of the hangtag label, including unique patient identifiers and cryobag identification information reported in CellChain™
    - If an error is identified, immediately contact Novartis Cell Therapy Operations. Do not attach an incorrect hangtag label to a cryobag

2. Precool the labeled hangtags by placing in dry ice or a freezer. Hangtag labels should be precooled for at least 10 minutes

Perform steps 3 and 4 during the first step in the cryobag/cassette packaging process:

3. Attach each precooled hangtag label to the corresponding cryobag. Novartis provides hangtag fasteners for this purpose
    - Ensure that the cryobag number on the hangtag label corresponds to the cryobag number on the institutional label
    - Hangtag labels must be attached to the cryobag
        - If institutional protocol requires cryobags to be cryopreserved inside an outer overlay bag, the hangtag label may be attached to the outer bag. If the overlay bag cannot accommodate the hangtag label, remove the overlay bag (as this is not required by Novartis), attach the hangtag label, and continue the packaging steps

4. Verify and document that the unique patient identifiers and cryobag information on the attached hangtag label match those on the institutional label

# T-Charge™ Leukapheresis Material Specifications

## Appendix Purpose

This appendix details clinical trial requirements for timing of leukapheresis, leukapheresis material specifications, and guidance on collection cell counts for manufacturing and Clinical Trials using the T-Charge™ platform.

Finished product "release for administration (RfA)" is noted in the last section of this appendix.

The T-Charge™ manufacturing platform utilizes devices with specific input and in-process control parameters. This platform allows the ex vivo culture time to be minimized and the finished product to be delivered earlier for patient treatment. Specific requirements and guidelines for leukapheresis material processed through the T-Charge™ platform are described below.

## Requirements for Timing of Leukapheresis Collection

- ✓ Cryopreserved leukapheresis material collected more than 30 months before the start of product manufacture date may **NOT** be shipped to Novartis without prior approval by Novartis Cell Therapy Operations
  - In case of questions, please contact Novartis Cell Therapy Operations

## Summary of Leukapheresis Material Specification Requirements

To be eligible for manufacturing, leukapheresis material must meet the specification requirements shown in Table 1.

For clinical trials, please consult your Clinical Trial Protocol for potential additional specifications.

**Table 1. Leukapheresis material specification requirements for T-Charge™**

| Specification | | Test Method/ Data Source | Acceptance Criteria |
|---|---|---|---|
| **Patient Infectious Disease Testing (Must be completed within 30 days of collection)[a]** | HBV, HCV, HIV | Autologous donor testing per institutional SOP using the health authority–approved test methods (Site-reported result) | Patient must not have HIV or active HBV or active HCV infection[a] |
| **Collection Cell Counts** | Viable TNC[b] | Per institutional SOP (Site-reported result) | ≥ 2.0 x $10^9$ TNCs[c] **Max 20.0 x $10^9$ TNC** |
| | Total number of viable CD3+ T cells[b] | Per institutional SOP (Site-reported result) | ≥ 1.0 x $10^9$ CD3+ cells[d] |
| | % T cells | Per institutional SOP (Site-reported result) | ≥ 5%[e] |
| **Cryobag** | Integrity and volume | Visual inspection upon thawing at Novartis facility | Intact bag/hermetically sealed ports **Max volume[f]: 300 mL** |

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SOP, standard operating procedure; TNC, total nucleated cells.

[a] Please see the Patient Infectious Disease Testing chapter for further details and ensure that any infectious disease testing timing requirements in your Clinical Trial Protocol are also met.

[b] Total cell counts are calculated by taking the sum of cell counts from multiple bags (if applicable).

[c] Total incoming (total nucleated cells) is recommended to not exceed 20.0 x $10^9$ TNC.

[d] For IMJ995, rounding rules may apply for pediatric patients only (≥ 1 x $10^9$ CD3+ T cells) to enable collections from lower weight (kg) patients. Please contact your Cell Therapy Operations Manager if specifications are not met.

[e] Percent (%) T cells will be autocalculated by CellChain™ based on site-reported data (Total CD3+ cells/TNC).

[f] All required cell counts **must** be fulfilled in a maximum of 300 mL cryopreserved leukapheresis material. **If total volume is greater than 300 mL, contact your Cell Therapy Operations Manager.**

# Collection Cell Counts

## *Requirements*

To be eligible for product manufacturing, collected leukapheresis material must meet minimum specification value requirements as outlined in Table 1. To ensure incoming leukapheresis material meets specifications for incoming material, and to maximize manufacturing success, careful attention must be given to the information in Table 1 and the guidance listed below.

Specification values are independent of the weight of the patient.

For clinical trials, please consult your Clinical Trial Protocol for potential additional requirements.

*Guidance*

- It is important to review the WBC differential of the collected leukapheresis material, including checking for the presence of tumor cells/blasts. Leukapheresis material containing high numbers of blasts or other non-CD3+ cells (eg, lymphoblasts, monocytes, etc.) may present challenges to T cell selection and cell expansion
    - It is recommended to report the WBC differential of the collected leukapheresis material, including the presence of blasts, in CellChain™
- Collections should target at least the minimum specification values, as described in Table 1 of this appendix

***Contact Novartis Cell Therapy Operations if you are unable to meet specifications.***

# Cell Processing

## *Volume Requirements*

- ✓ In order to meet the volume limitation (final cryoformulated volume: 300 mL), the Novartis-preferred process is to reduce **volume and add cryoprotectant solution in order to achieve the recommended target cell concentration of 0.5 x $10^8$ - 2.5 x $10^8$ WBC/mL**
- ✓ If any assistance is needed, please contact your Cell Therapy Operations Manager

## *Requirements for Sentinel (QC) Vials*

- ✓ Sentinel (QC) vials must be 1- to 2-mL cryovials approved for storing cells at cryogenic temperatures
- ✓ Sentinel (QC) vials must be filled with ≥1 mL of leukapheresis material **with the same cryoformulation and WBC concentration as that in the cryobags**
- ✓ Collect up to 5 sentinel (QC) vials:
    - One (1) vial will be stored at your site per institutional SOPs at least until delivery of finished product
    - Two (2) to four (4) vials will be shipped with the cryopreserved material. Four (4) shipped vials are preferred, but it is acceptable to collect and ship fewer vials—at least 2 vials—in cases with low cell counts

***Note**: Additional vials may be required and possibly shipped to a different location for clinical trials. (Refer to your T-Charge™ Clinical Trial Protocol and or/central lab manual for a description of additional samples to be collected. Contact your Novartis Clinical Research Associate in case of questions.)*

- ***In cases of low cell counts, the vials to the manufacturing facility should be prioritized***

## Finished Product "Release for Administration (RfA)" Based on Interim Sterility Results

- Finished product will be released for administration based on interim sterility results (negative-to-date sterility test readout with 3 days' incubation)
- This allows rapid release shipment of the product (during which the final sterility testing is completed)
- The risk of transmission of infectious agents associated with finished product (estimated to be about 0.1% based on historical sterility test failures) remains low with this interim negative-to-date sterility test approach and may benefit patients who may require immediate access to therapy
- It is up to the discretion of the investigator to treat patients with 3-day interim sterility released product before the final sterility results become available

# Change History for Global Leukapheresis Reference Manual Version G3.1

| Chapter/Section | Previous Version: Global G3 | Current Version: Global G3.1 | Justification |
|---|---|---|---|
| **Introductory Changes** | | | |
| Introduction | Included contact numbers for Europe in general and France specifically. | Updated contact information for Europe (Commercial-except France), France (Commercial), and EU Clinical. | To provide corrected contact information for both commercial and clinical orders. |
| Table of Contents | Chapter titled "Leukapheresis Material Specifications". | Chapter renamed to "CTL019/Tisagenlecleucel Leukapheresis Material Specifications". | To differentiate from the new T-Charge™ leukapheresis material specifications, which are presented in the Appendix 4. |
| Table of Contents | N/A | Updated to include Appendix 4: T-Charge™ Leukapheresis Material Specifications. | To align with new content. |
| Chain of Identity | Definition indicated that the patient's middle name or initial of middle name was optional. | Clarified that the initial of the middle name is optional. | To clarify COI definition. |
| **CTL019/Tisagenlecleucel Leukapheresis Material Specifications** | | | |
| Chapter Purpose | N/A | Clarified that specifications in this chapter are for CTL019/tisagenlecleucel and that T-Charge™ specifications are presented in Appendix 4. | To differentiate specifications for CTL019/tisagenlecleucel from T-Charge™. |
| Summary of Leukapheresis Material Specification Requirements | Referred to all clinical trials. | Clarified as "CTL019 clinical trials". | To differentiate from specifications for T-Charge™, which are presented in Appendix 4. |
| Summary of Leukapheresis Material Specification Requirements | Table 1 listed leukapheresis material specification requirements. | Clarified that the specification requirements in Table 1 of this chapter pertain to CTL019/tisagenlecleucel. | To differentiate from specifications for T-Charge™, which are presented in Appendix 4. |
| **Precollection: Determining Patient Readiness for Leukapheresis** | | | |
| Initial Health Assessment | Referred to the "Leukapheresis Material Specification" chapter for further details. | Clarified as the "CTL019/Tisagenlecleucel Leukapheresis Material Specification" chapter and also added a reference to "Appendix 4: T-Charge™ Leukapheresis Material Specifications" for further details. | To differentiate chapters and also include reference to new T-Charge requirements and guidance. |

| Chapter/Section | Previous Version: Global G3 | Current Version: Global G3.1 | Justification |
|---|---|---|---|
| **Leukapheresis Collection** | | | |
| Determining Patient Readiness for Leukapheresis | Referred to the "Leukapheresis Material Specification" chapter for further details. | Clarified as the "CTL019/Tisagenlecleucel Leukapheresis Material Specification" chapter and also added a reference to "Appendix 4: T-Charge™ Leukapheresis Material Specifications" for further details. | To differentiate chapters and also include reference to new T-Charge requirements and guidance. |
| Guidance on Peripheral Blood Testing | Referred to the "Leukapheresis Material Specification" chapter for specification requirements. | Clarified as the "CTL019/Tisagenlecleucel Leukapheresis Material Specification" chapter and also added a reference to "Appendix 4: T-Charge™ Leukapheresis Material Specifications". | To differentiate from specifications for T-Charge™, which are presented in Appendix 4. |
| Leukapheresis Collection | Referred to Terumo BCT, Inc document #361 for further guidance on collection optimization using the Spectra Optia®. | Removed reference to the additional document. | Document is no longer in use by Terumo BCT, Inc. |
| **Leukapheresis Material Transfer and Testing** | | | |
| Leukapheresis Material Testing | Table 1 listed leukapheresis material specification requirements and the "Leukapheresis Material Specifications" chapter was referred to for further guidance. | Clarified that the specification requirements in Table 1 in this chapter pertain to CTL019/tisagenlecleucel.<br><br>Clarified as the "CTL019/Tisagenlecleucel Leukapheresis Material Specification" chapter and also added a reference to "Appendix 4: T-Charge™ Leukapheresis Material Specifications". | To differentiate from specifications for T-Charge™, which are presented in Appendix 4. |
| **Leukapheresis Cell Processing** | | | |
| Cell Processing | N/A | Added a note to see Appendix 4 for T-Charge™ volume specifications. | To highlight new volume requirements for T-Charge™. |
| Requirements for Sentinel (QC) Vials | N/A | Added a bullet clarifying that for clinical trials vials for manufacturing should be prioritized. | To clarify requirements for sentinel (QC) vials. |

| Chapter/Section | Previous Version: Global G3 | Current Version: Global G3.1 | Justification |
|---|---|---|---|
| Documentation of the Cell Processing Procedure | Requirement for cryopreservation date/time to be noted and entered into CellChain™. | Included the definition of cryopreservation date/time. | Clarification of reporting requirement. |
| **Appendix 2: Packing and Shipping: evo® DV10 Dry Vapor Shipper** | | | |
| All chapter sections | Referred to the "Shipper Certification Form". | Updated language to refer to the "evoIS DV10 Certified Shipment Report". | To clarify the name of required documentation for the evo® DV10 shipper. |
| **Appendix 4: T-Charge™ Leukapheresis Material Specifications** | | | |
| All chapter sections | N/A | Added a new Appendix detailing the clinical trial requirements for timing of leukapheresis, leukapheresis material specifications, and guidance on collection cell counts for manufacturing and clinical trials using the T-Charge™ platform. | To provide requirements and guidance for new clinical trials using the T-Charge™ platform. |

# Signature Page

# For the Novartis Leukapheresis Reference Manual

## CAR-T

**(KYMRIAH/Tisagenlecleucel/CTL019 and clinical CAR-T cell products)**

## Version G3.1

This signature page is used to record the approval of the Novartis Leukapheresis Reference Manual: CAR-T (KYMRIAH/Tisagenlecleucel/ CTL019 and clinical CAR-T cell products) vG3.1.

The approvals are appended as Novartis PKI electronic signatures.

| Name/Title | Signature |
|---|---|
| *Author*<br>**Lisa Keeler**<br>Cell Therapy Operations Manager; OBU | Keeler Lisa<br>Digitally signed by Keeler Lisa<br>DN: dc=com, dc=novartis, ou=people, ou=PH, serialNumber=1932737, cn=Keeler Lisa<br>Reason: I have reviewed this document<br>Date: 2022.09.16 15:28:31 -04'00' |
| *Author*<br>**Anne Lecat**<br>Cell Therapy Operations Manager; OBU | Lecat Anne<br>Digitally signed by Lecat Anne<br>DN: dc=com, dc=novartis, ou=people, ou=TO, serialNumber=713005, cn=Lecat Anne<br>Reason: I have reviewed this document<br>Date: 2022.09.19 09:17:14 +02'00' |
| *Reviewer*<br>**Lee Clough**<br>Regional Director, Cell Therapy Operations; OBU | Clough Lee<br>Digitally signed by Clough Lee<br>DN: dc=com, dc=novartis, ou=people, ou=PH, serialNumber=2074360, cn=Clough Lee<br>Reason: I am approving this document<br>Date: 2022.09.20 07:14:13 -04'00' |
| *Reviewer*<br>**Cathy Afable**<br>Regional Director, Cell Therapy Operations; OBU | Cifariello Annamaria<br>Digitally signed by Cifariello Annamaria<br>DN: dc=com, dc=novartis, ou=people, ou=PH, serialNumber=1793620, cn=Cifariello Annamaria<br>Reason: I have reviewed this document<br>Date: 2022.09.19 15:29:11 +02'00' |
| *Reviewer*<br>**Annamaria Cifariello**<br>Regional Director, Cell Therapy Operations; OBU | Afable Cathy<br>Digitally signed by Afable Cathy<br>DN: dc=com, dc=novartis, ou=people, ou=TO, serialNumber=1751882, cn=Afable Cathy<br>Reason: I have reviewed this document<br>Date: 2022.09.20 10:13:44 +08'00' |
| *Reviewer*<br>**Jaclyn Davis**<br>Sr. Clinical Development Medical Director; C&G Clinical (C&G) | Davis Jaclyn<br>Digitally signed by Davis Jaclyn<br>DN: dc=com, dc=novartis, ou=people, ou=GD, serialNumber=1981994, cn=Davis Jaclyn<br>Date: 2022.09.16 16:01:38 -04'00' |
| *Approver*<br>**Bernhard Hillenbrand**<br>Global Product Lead; MS&T/Technical Services CGT | Hillenbrand Bernhard<br>Digitally signed by Hillenbrand Bernhard<br>DN: dc=com, dc=novartis, ou=people, ou=TO, serialNumber=1778464, cn=Hillenbrand Bernhard<br>Reason: I am approving this document<br>Date: 2022.09.26 19:01:36 +02'00' |
| *Approver*<br>**Lisa Sweterlitsch**<br>Apheresis QA Lead Americas; NTO-CGT | Sweterlitsch Lisa<br>Digitally signed by Sweterlitsch Lisa<br>DN: dc=com, dc=novartis, ou=people, ou=TO, serialNumber=1661307, cn=Sweterlitsch Lisa<br>Reason: I am approving this document as the Aph QA Regional Lead<br>Date: 2022.09.26 14:22:47 -04'00' |

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