D5.2.2 *From the genetic mother including egg donors, if possible, suitable material for preparation of at least 50 μg genomic DNA.*

GUIDANCE:
Material suitable for preparation of genomic DNA may be purified DNA, frozen cellular material, or blots. The CBB must validate that the volume of the sample is adequate to prepare at least 50 μg.

STANDARD:
D6 CRYOPRESERVATION

D6.1 *CB units shall be cryopreserved using controlled rate freezing or an equivalent procedure. If an equivalent procedure is used, it shall be validated to maintain equivalent recovery and viability of nucleated cells.*

GUIDANCE:
Although controlled rate freezing by use of a programmable device is the recommended method for cryopreserving CB units, alternative methods validated to ensure cell viability may be used. Validation should include viability or potency tests required in D10. Other methods of freezing, for example, freezing in a mechanical freezer, can be acceptable with the appropriate level of vigilance:

- Ensure that canisters are separated and not stacked, to allow airflow around each unit.
- Limit access to the freezer during the freezing process so the temperature within the interior is not compromised.
- Trace the freezing kinetics via a data logger or other alternative tracing device to produce a cooling curve that demonstrates acceptable execution.

Acceptable ranges for t

D6.2 בתהליך סטנדרטי של שימור קריוגני יש לתעד את המידע הבא עבור כל מנה:
D6.2.1 ריכוז אוכלוסיית התאים המגורענים בטווח המוגדר.

STANDARD:

D6.2 *Cryopreservation Standard Operating Procedures shall specify that the following information is recorded for each unit:*

D6.2.1 *Total nucleated cell concentration within a defined range.*

D6.2.2 *The cryoprotectant, its final concentration, and the duration of cell exposure prior to freezing.*

D6.2.3 *Method of freezing and end-point temperature of cooling.*

D6.2.4 *Cooling rate within a defined range.*

D6.3 יש לשמור מנות דם טבורי בשקיות הקפאה שעוצבו ואושרו עבור שימור של תאים ממקור אנושי בהקפאה...

D6.3 *CB units shall be stored in freezing bags designed and approved for the cryopreservation of human cells and shall be placed into metal canisters to provide protection during freezing, storage, transportation, and shipping.*

שימוש במבחנות למטרת שימור ארוך טווח איננו קביל היום.

GUIDANCE:

The use of vials to provide long-term storage is not acceptable at this time. While it is possible that future innovation may modify this standard, the use of vials has a possibility to increase contamination, and may be difficult to prepare for infusion in the Clinical Program. CB units stored in vials prior to these Standards can still be used; however, the CBB should inform the Clinical Program so that the appropriate planning for storage and infusion can take place.

STANDARD:

D6.3.1 *Each freezing bag and its satellite container(s), if any, shall be examined visually for damage or possible contamination prior to use.*

D6.3.2 *Freezing bags shall allow the filling of at least two contiguous segments.*

D6.3.2 שקיות הקפאה צריכות לאפשר מילוי של שני סגמנטים צמודים לפחות.

GUIDANCE:

Segments must [illegible]it in the event the segment becom[illegible]tached during the cryopreservation [illegible] it belongs to and which staff member identified the sample. One way to comply with this standard is through the use of labels or stickers; however, the CBB may decide how to identify the sample as long as it accurately traces it to the CB unit.

STANDARD:

D6.4 *After filling, each freezing bag shall be visually examined for possible leaking, overfilling or underfilling of the freezing bag, and breakage of seals. The results of these inspections shall be documented.*

GUIDANCE:

Overfilling is defined as exceeding the manufacturer's volume recommendations. Underfilling can be equally detrimental to product safety as bags would be thinner, more brittle, and particularly susceptible to breakage. Exposure of product to nitrogen, whether liquid or vapor phase, is in itself a hazard because liquid nitrogen is not sterile. Aerosols are created in the vapor phase above the liquid when materials warmer than -196°C are introduced into the liquid.

The inspector should ensure that the CB Processing Facility's policies include guidelines to remove air and prevent overfilling bags. These issues have resulted in broken seals and bags that explode as the bag rapidly expands when exposed to warmer temperatures, even that of nitrogen vapor.

STANDARD:

D6.5 *The duration from addition of cryoprotectant to initiation of freezing shall be minimized and validated by the CBB.*

GUIDANCE:

A CBB's policies should emphasize attempts to minimize the time between addition of DMSO and initiating cryopreservation. This can also be included as a key element in staff training and evaluated at the time of competency assessment. Validation studies should be performed that includes how many CB units can be simultaneously processed within a timeframe that maintains cell viability.