

CBMTG Laboratory Committee-Laboratory Guidance

Summary of Validation of Cryopreservation Bags

The objective of this validation was to confirm, by examination and objective evidence, that a selected cryopreservation bag is compatible with institutional policies and standard operating procedures plus functions adequately so as not to impact final product integrity. Bags from 3 different manufactures were tested by 6 facilities across Canada;

- Origen Biomedical Cryostore EVA 500ml (mfg # CS500n)*
- Origen Biomedical Cryostore EVA 250ml (mfg # CS250n)*
- Maco Biotech Freezing-EVA Bags 60 - 100ml (mfg # GSR5001AU)**
- Maco Biotech Freezing-EVA Bags 30 -70ml (mfg # GSR2001AU)**
- Miltenyi Biotech CryoMACS® Freezing Bag 500 (mfg # 200-074-407)
- Miltenyi Biotech CryoMACS® Freezing Bag 250 (mfg # 200-074-406)

* Origen bags validated prior to release of upgraded bags; Technical bulletin 0810

**Maco Biotech bag nomenclature presents the fill volume instead of the nominal volume.

Compatibility Assessment

Participants reviewed the manufacturer's product insert/instructions for use and certificate of analysis plus observed the bags to evaluate compatibility with their laboratory and infusion site policies, standard procedures and ancillary supplies (i.e. transfer sets, syringes, freezing cassettes, infusion sets etc.). Situations where facilities would need to deviate from manufacturers instructions and other potentially pertinent observations are summarized below. Comments which were repeated are followed by the number of participants in brackets.

	Deviations	Comments
Origen 250 ml 1 participant		<ul style="list-style-type: none"> • Printing on label pocket required careful placement of label to prevent obstruction. • Needle free port required simple modification to process. • Comfortable to work with. • Easy to remove excess air. • Slits in port protectors allowed for complete drainage
Maco 30 -70 ml 1 participant		<ul style="list-style-type: none"> • Bags too large for cassettes (tall port covers) • 4 lines available for use with clamps instead of rollers • Difficult to degas. • Tubing and pocket difficult to seal. • Difficult to obtain samples from either sampling port or transfer line • Unable to access bags with current facility infusion supplies.
Miltenyi 250 ml 1 participant		<ul style="list-style-type: none"> • Very similar to bags currently in use • Couldn't access sampling port with a 16g x 1 1/2 needle. • Excess air could not be removed with out uncapping a line. • Port design is difficult to use with current facility supplies • Unable to access bags with current facility infusion supplies.

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	Deviations	Comments
<p>Origen 500 ml</p> <p>5 participants</p>	<ul style="list-style-type: none"> The Mfg instructions require cutting sealed tubing so that the end of the stub is below the spike port tops. This helps prevent damage from abrasion by the cassettes. Current facility procedure requires 2 seals on the tubing after aliquoting which resulted in a stub which extended beyond the spike ports. Mfg instructions state that labels should not be applied directly to the bag. Current facility labelling includes applying regulated labels to the bag surface. 	<ul style="list-style-type: none"> Needle free injection port. No over wrap provided. Tubing closest to the bag (approx. 15mm) is thicker than the remainder of the tubing and could not be sealed effectively. Sealing after this section leaves tails that are snug to the cassette (X2). Spike protectors cause tight fit in cassettes (X2). Difficult to remove all bubbles due to long spiking guards (x2). Full lot number is not imprinted on the bag as it appears on the wrapper. Close proximity of infusion ports makes accessing awkward. Infusion port tabs are difficult to remove. Drained well. Difficult to drain completely due to large spike protectors. Incompatible with current sampling site couplers used for infusion by syringe.
<p>Maco 60 - 100 ml</p> <p>5 participants</p>		<ul style="list-style-type: none"> Certificate of analysis and instructions for use must be obtained from mfg. No over wrap provided (x2). Sterile packaging. Label pocket fits labels without folding. Label pocket too large. Label pocket is at the bottom of the bag therefore does not show when product is in metal presses. Fits current metal presses and cassettes. Tight fit in cassettes. Seams on all sides of bag: breakage risk? Injection port is situated perpendicular to the tubing limiting needle access (X2) 4 transfer lines all with different connector types not be compatible facility processes (X2). Difficult to degas. Tubing by bag takes longer to seal (X3). Bag dimension results in a thicker aliquot which was quite noticeable during thawing. Incompatible with current sampling site couplers used for infusion by syringe. Easy to spike. Long spike guards with splits to allow cells to drain. Good drainage.
<p>Miltenyi 500 ml</p> <p>5 participants</p>	<ul style="list-style-type: none"> Mfg instructions require use of over wrap bags during freezing and storage. Current facility procedures do not include the use of over wrap bags (2). Mfg instructions require transfer to LN2 vapour for at least 4 hours after storage in liquid phase LN2. Facility processes do not include this step. 	<ul style="list-style-type: none"> Individual packaging. Comes with over wrap. Lot number is imprinted on bag but not expiry date. Packaging difficult to open. Injection/sampling port may be restrictive for some needle sizes (X2). Mfg requirement to transfer liquids containing DMSO only through the injection port may not be compatible with some facility processes. Difficult to degas. Spike ports are tight (x2). Good drainage. Incompatible with current sampling site couplers used for infusion by syringe. Product was not licensed as a Medical Device with Health Canada as of 2010-05-05 (x3).

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Freeze/Store/Thaw Test

Bags were frozen, stored and thawed following standard facility processes. Minimum and maximum fill volumes were challenged. The processes were repeated for a total of 3 freeze/store/thaw cycles on each bag tested. Critical process data, such as actual storage and thawing temperatures were documented by individual facilities. No significant process deviations were reported.

Allowable Ranges

Facility #	Pre storage Temp	Storage Temperature	Transport Temperature	Thaw Temperature
1	≤ -60°C	≤ - 150 °C	≤ - 150 °C	35 - 40 °C
2	-90 °C	≤ - 150 °C	≤ - 150 °C	37 - 40 °C
3	-90 °C	-120 °C to -196 °C	-120 °C to -196 °C	38 - 40 °C
4	-90 °C	≤ - 180 °C	≤ - 185 °C	37 - 42 °C
5	-100 °C	-150 °C to -196 °C	-120 °C to -150 °C	35 - 39 °C
6	< -130°C	< -130°C	< -130°C	36 - 38 °C

Origen Biomedical Cryostore EVA 250ml (mfg # CS250n)

	CRYO SOLUTION		FREEZING		STORAGE		TRANSPORT		THAW	
	10% DMSO	other	uncontrolled	controlled	liquid phase LN2	vapour phase LN2	dry shipper	other	water bath	other
# of bags tested	14	0	0	14	0	14	14	0	14	0
# of failures	0	0	0	0	0	0	0	0	0	0

Maco Biotech Freezing-EVA Bags 30 -70ml (mfg # GSR2001AU)

	CRYO SOLUTION		FREEZING		STORAGE		TRANSPORT		THAW	
	10% DMSO	other	uncontrolled	controlled	liquid phase LN2	vapour phase LN2	dry shipper	other	water bath	other
# of bags tested	14	0	0	14	0	14	14	0	14	0
# of failures	0	0	0	0	0	0	0	0	0	0

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Miltenyi Biotech CryoMACS® Freezing Bag 250 (mfg # 200-074-406)

	CRYO SOLUTION		FREEZING		STORAGE		TRANSPORT		THAW	
	10% DMSO	other	uncontrolled	controlled	liquid phase LN2	vapour phase LN2	dry shipper	other	water bath	other
# of bags tested	14	0	0	14	0	14	14	0	14	0
# of failures	0	0	0	0	0	0	0	0	0	0

Origen Biomedical Cryostore EVA 500ml (mfg # CS500n)

	CRYO SOLUTION		FREEZING		STORAGE		TRANSPORT		THAW	
	10% DMSO	5% DMSO	uncontrolled	controlled	liquid phase LN2	vapour phase LN2	dry shipper	other	water bath	other
# of bags tested	24	2	2	24	8	18	26	0	26	0
# of failures	0	0	0	0	0	0	0	0	0	0

Maco Biotech Freezing-EVA Bags 60 - 100ml (mfg # GSR5001AU)

	CRYO SOLUTION		FREEZING		STORAGE		TRANSPORT		THAW	
	10% DMSO	other	uncontrolled	controlled	liquid phase LN2	vapour phase LN2	dry shipper	other	water bath	other
# of bags tested	24	2	2	24	8	18	26	0	26	0
# of failures	0	0	0	0	*3	0	0	0	0	0

*leaking from end of segment after cycle 3 (seal broke after cycle 2 but didn't leak)

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Miltenyi Biotech CryoMACS® Freezing Bag 500 (mfg # 200-074-407)

	CRYO SOLUTION		FREEZING		STORAGE		TRANSPORT		THAW	
	10% DMSO	other	uncontrolled	controlled	liquid phase LN2	vapour phase LN2	dry shipper	other	water bath	other
# of bags tested	24	2	2	24	8	18	26	0	26	0
# of failures	0	0	0	0	0	0	0	0	0	0

Participant contact information:

Angeline Giftakis, agiftakis@exchange.hsc.mb.ca

Brenda Letcher, brenda.letcher@blood.ca

Giovanna Cameron, GCameron2@bccancer.bc.ca

Mike Halpenny, mike.halpenny@blood.ca

Pamela O'Hoski, ohoskpam@hhsc.ca

Susan Berrigan, susan.berrigan@cls.ab.ca