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Freezing Media Comparison Study Using CryoStor® CS10

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The Study

Comparison of our current freezing method versus using commercially available CryoStor® CS10 for cryopreservation of QC vials of our allogeneic patient products.

Samples

10 total Hematopoietic Progenitor patient samples were collected. 5 Apheresis products and 5 Marrow products.

Method

- Current freezing method: 500mL freezing media, expires one month. 10mL freezing solution expires 24 hours. 2mL from each solution was added to each product sample and aliquoted into 4 vials.
- 2 additional vials were frozen using CryoStor® CS10.
- 2 vials from each method were thawed and tested for WBC counts, trypan blue viability, and CD34 7AAD viability.
- 5 of the patient products had CFUs plated for informational purposes only.

Acceptance Criteria

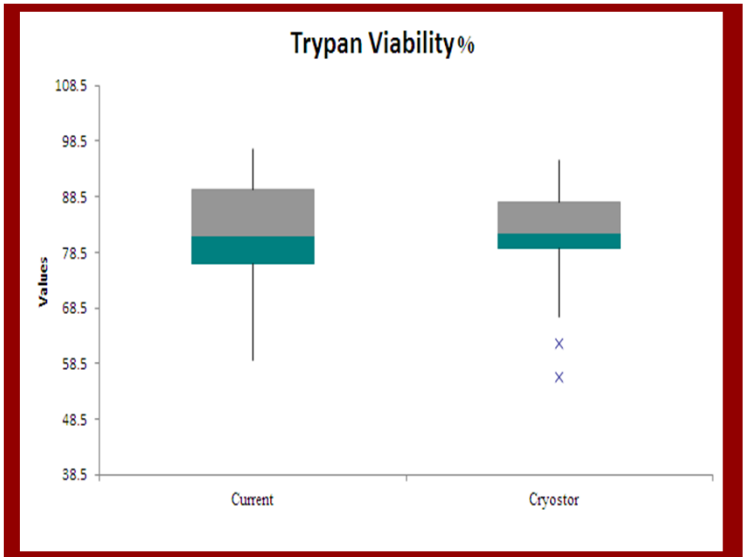
- Trypan blue viability above 60%
- Trypan blue viability within 30%
- CD34 7AAD viability within 30%

Results

- All 10 sets of patient samples had trypan blue viability above 60% with the exception of patient 9*.
- All 10 sets had trypan blue viability within 30%.
- All 10 sets had CD34 7AAD viabilities within 30%.
- Cannot reject the null hypothesis p>0.05 therefore we can conclude the means are the same for both Trypan viability and CD34 7AAD Viability.

Trypan Viability%

Sample	Product Type	Current	Cryostor	Viability ≥60% Y/N	Within 30% Y/N
1	HPCA	78	62	Y	Y
2	HPCM	76	86	Y	Y
3	HPCA	75	79	Y	Y
4	HPCA	95	91	Y	Y
5	HPCA	89	84	Y	Y
6	HPCM	90	88	Y	Y
7	HPCA	83	80	Y	Y
8	HPCM	80	80	Y	Y
9	HPCM	59	56	N*	Y
10	HPCM	97	95	Y	Y
	Average	82.2	80.1	P-value: 0.652	

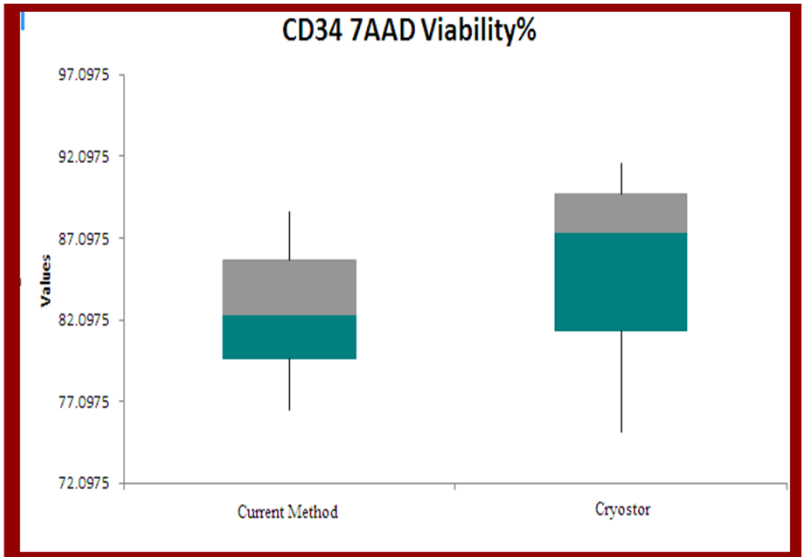


Labor and Supply Cost Comparison

- Freezing Media: $112.87 (Time: 2 hours)
- Freezing Solution: $64.26 (Time: 30 mins)
- CryoStor® CS10 : $63 (Time: 10 mins)

CD34 7AAD Viability%

Sample	Product Type	Current	Cryostor	Within 30%? Y/N
1	HPCA	82	91.7	Y
2	HPCM	85.7	86.4	Y
3	HPCA	77.9	86.1	Y
4	HPCA	82.8	90.8	Y
5	HPCA	79.7	76.4	Y
6	HPCM	88.7	88.7	Y
7	HPCA	79.8	79.9	Y
8	HPCM	76.6	88.6	Y
9	HPCM	85.7	75.6	Y
10	HPCM	87.4	90.1	Y
	Average	82.6	85.4	P-value: 0.876



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- QA (Mary Ann Kelley, Michelle Lynch, Eric Hubbard)
- **CryoStor® CS10: https://www.stemcell.com/cryostor-cs10.html**

Comparison study of current freezing media versus Cryostor 10

For this validation, we compared our current freezing medias to Cryostor10 in order to determine the efficacy of this commercially available product for QC vial cryopreservation. We collected 10 allogeneic Hematopoietic Progenitor Cell patient products, 5 HPC Apheresis and 5 HPC Marrow. Our current method consists of freezing four nunc vials each with $15x10^6$ cells for HPC Apheresis and $30x10^6$ cells for HPC Marrow using 2mL of premade freezing solution (RPMI, FBS, Hepes Buffer, and Penicillin) in combination with 2mL freezing media (RPMI, DMSO, EDTA and Sodium Bicarbonate). In this validation 2 additional vials of cells were cryopreserved in 2mL of CryoStor10 post processing alongside control vials which were prepared using our current approved freezing method. The vials were stored overnight in a -80 freezer and then transferred to LN2 storage.

At a later date, we thawed two vials each from cells frozen the current way and the two vials frozen with CryoStor10. Each pair of sample vials were thawed in a water bath at 37 degrees Celsius and then pooled into a 15mL conical. Samples were then transferred to QC for WBC counts, trypan blue viability and CD34 7AAD. CFU's were plated on 5 samples for informational purposes only. For this validation, testing parameters required the trypan blue to be above 60% and each sample within 30% of one another. The CD34 7AAD also had to be within 30% of one another.

While comparing our current cryopreservation method of allogeneic cells in vials versus using Cryostor10, all trypan blue viabilities fell within 30% of one another, and all CD34 7AAD viabilities fell within 30% of one another. One set of samples fell below 60% trypan blue viability but were comparable and were within 3% of each other. CFU's were plated for information only, but showed comparable results. Stability results from an opened bottle of Cryostor demonstrated stable results after a 10-day period. For sterility, 10ml injection vials will be used in order to reduce the risk of contamination to the product. Cryostor 10 is a valid method for freezing allogeneic QC cells as it yielded comparable results to that of the current method.