Evaluation of Gamma Irradiated and Limited Donor Pool Human Platelet Lysate for Clinical Cell Manufacturing

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Background

- The use of fetal bovine serum (FBS) for clinical manufacturing of cell therapy products poses risks, including the potential for viral and prion transmission and the possibility of adverse immunological reactions.
- Human platelet lysate (hPL) has emerged as a viable, xenogeneic-free alternative to FBS in all steps of cell manufacturing.
- Differences in hPL manufacturing processes can significantly impact stem cell growth, morphology, and multipotency. To address these issues, Compass Biomedical has developed a highly standardized, industrial-scale production process for our PLUS™ hPL using good manufacturing practices (GMP).
- European Pharmacopoeia (Ph. Eur.) 9th Edition 5.2.12: *"Because of the inherent risk of transmitting infectious agents from pooled plasma, pooled sera, or other derivatives from pooled allogenic human blood or plasma, consideration is given to limit the number of donations which are pooled, unless sufficient methods for inactivation/removal of viruses are applied during production, where applicable."*

Infectious Disease Screening

- Each platelet unit used in the manufacturing of PLUS™ hPL has been screened for infectious diseases at AABB accredited Blood Banks using FDA approved methods and is suitable for transfusion.
- Inherent risk of transmitting infectious agents remains a concern for allogeneic blood or plasma products.
- Unknown pathogens cannot be screened at the time of blood collection.
- Gamma irradiation at 25-40 kGy is commonly applied to FBS for pathogen reduction purposes.

Infectious Disease	Test Method
Human Immunodeficiency Virus	• HIV I/II Ab • HIV-NAT
Human T-Lymphotropic Virus	• HLTV I/II Ab
Hepatitis B Virus	• HBs Ag • HBc Ab • HBV-NAT
Hepatitis C Virus	• HCV Ab • HCV-NAT
Syphilis	• RPR or FTA-ABS
West Nile Virus	• WNV-NAT
Trypanosoma cruzi	• T. cruzi Ab

Methods

Gamma irradiation of three different lots of GMP PLUS™ was performed in the dose range of 25-40 kGy. The samples were kept frozen on dry ice throughout shipping and irradiation. Three lots of limited donor PLUS™ were also evaluated. Limited donor PLUS™ consists of pooling only 15 platelet apheresis units instead of the minimum 100 units. The gamma irradiated and limited donor PLUS™ were then evaluated in terms of physiochemical profile (pH, osmolality, and total protein concentration), concentrations of important growth factors (VEGF, EGF, FGF-basic, and PDGF-BB), and ability to promote growth of bone marrow-derived mesenchymal stromal cells (MSCs).

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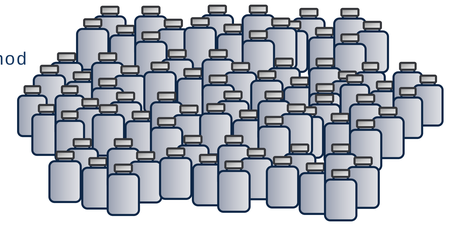
Comparison of Pathogen Reduction Methods

Gamma Irradiated hPL

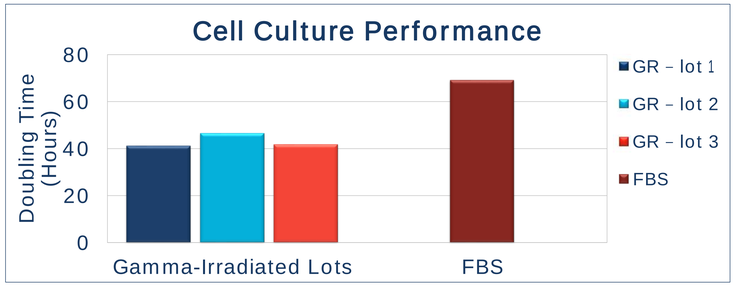
- Reduces risk by using a pathogen reduction method
- Lot sizes can be scaled to over 100 L
- Large hPL lots are more suitable for use as an ancillary material for large scale cell therapy manufacturing and other applications.



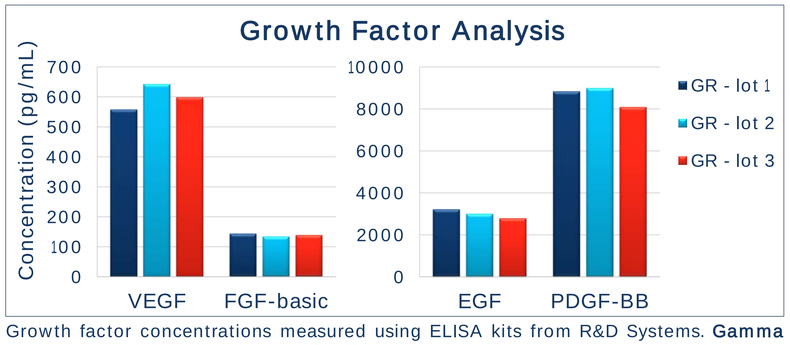
Limited Donor hPL

- Reduces risk but does NOT include any pathogen reduction
- Limiting to 15-donor pools results in small hPL lots of approximately 3 L each.
- Limited Donor hPL lots are more suitable for use as an ancillary material for small scale or autologous cell therapy manufacturing. Small lot sizes result in a more costly process for large-scale cell therapy manufacturers.

Gamma Irradiated PLUS™ hPL



Data represents the doubling time of P3 hMSCs (n=1) cultured in αMEM supplemented with three different lots of Gamma Irradiated (25-40 kGy) PLUS™ hPL (all at 10% v/v). **Culture of hMSCs with Gamma Irradiated PLUS™ resulted in decreased doubling times when compared to hMSCs cultured in MSC-qualified FBS (10% v/v).**

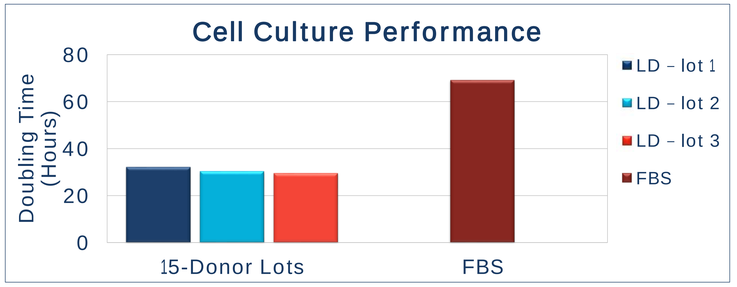


Growth factor concentrations measured using ELISA kits from R&D Systems. **Gamma irradiation (at 25-40 kGy) of PLUS™ hPL did not compromise consistency of growth factor content between lots.**

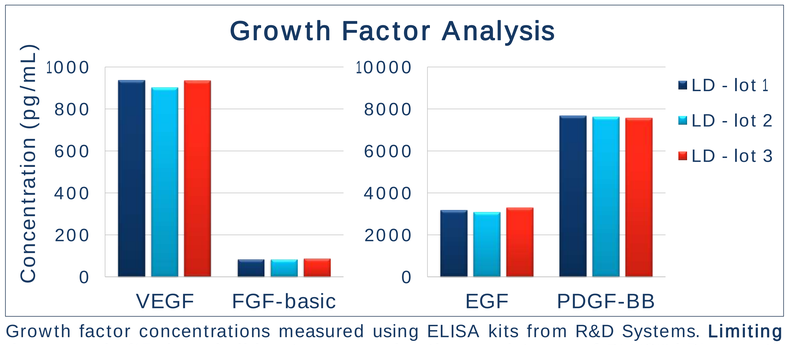
The physiochemical profile (pH, osmolality, and total protein content) of three different lots of gamma irradiated PLUS™ were compared. Gamma irradiation did not have a significant effect on lot-to-lot consistency.

	pH	Osmolality (mOsm/kg)	Total Protein (g/dL)
GR-lot 1	7.0	326	5.12
GR-lot 2	7.0	340	4.50
GR-lot 3	7.1	326	5.05
Avg.	7.0	331	4.89
StDev	0.04	8.08	0.34

Limited Donor PLUS™ hPL



Data represents the doubling time of P3 hMSCs (n=1) cultured in αMEM supplemented with three different lots of 15-Donor PLUS™ hPL (all at 10% v/v). **Culture of hMSCs with 15-Donor PLUS™ resulted in decreased doubling times when compared to hMSCs cultured in MSC-qualified FBS (10% v/v).**



Growth factor concentrations measured using ELISA kits from R&D Systems. **Limiting donor pools (15-donor) did not compromise the consistency of PLUS™ hPL growth factor content between lots.**

The physiochemical profile (pH, osmolality, and total protein content) of three different lots of limited donor PLUS™ were compared. Limiting donor pools (15- donors) did not have a significant effect on lot-to-lot consistency.

	pH	Osmolality (mOsm/kg)	Total Protein (g/dL)
LD-lot 1	7.2	340	5.06
LD-lot 2	7.0	355	4.59
LD-lot 3	7.1	350	4.51
Avg.	7.1	342	4.72
StDev	0.10	7.64	0.29

Conclusions

- Both gamma irradiation of industrial-scale PLUS™ lots and limited donor pools (15 donors) do not compromise consistency or performance of PLUS™
- While both gamma irradiation and limited donor pooling reduce the risk of viral transmission, gamma irradiation is a preferred pathogen reduction method for hPL due to:
  - High penetration depth
  - Easily adopted into the GMP process
  - More cost-effective for end users than limited donor hPL
- Release date of GMP Gamma Irradiated hPL (PLUS™-GR): Q3 2018
- Future Plan: Complete a viral inactivation study to validate that the dose range of our GMP Gamma Irradiated hPL (PLUS™-GR) results in a 4-fold $\log_{10}$ reduction of viral contamination.