Irradiation Methods for Pathogen Reduction of Xeno-Free Human Platelet Lysate

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Human Platelet Lysate

- The use of fetal bovine serum (FBS) for clinical manufacturing of stem cell 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 in all steps of cell manufacturing.
- Traditionally, hPL has been prepared by individual laboratories in small batches using protocols that differ in the number of platelet units pooled, the processing of platelets, and the requirement for heparin. These differences 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).

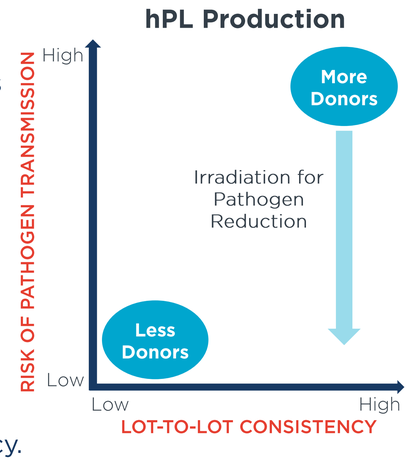
Infectious Disease Screening

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.

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

Concerns with Unknown Pathogens

- 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.
- The risk of transmitting infectious agents increases when a larger number of platelet apheresis donations are pooled.
- A smaller donor size will compromise hPL lot-to-lot consistency.
- **Solution:** Irradiate hPL consisting of large donor pools for complete pathogen reduction while preserving lot-to-lot consistency.



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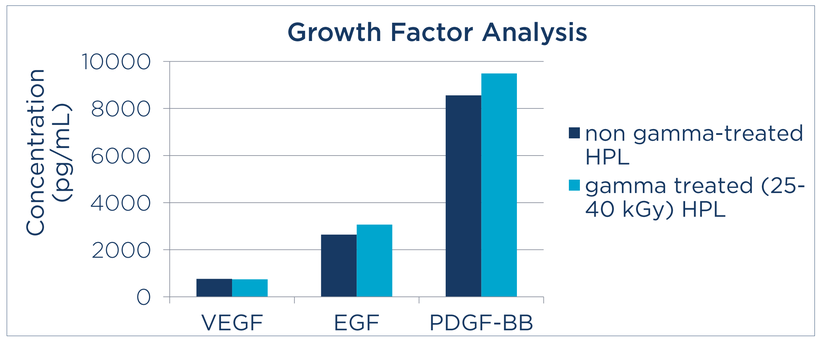
Comparison of Pathogen Reduction Methods

- Gamma Irradiation at 25-35 kGy is commonly applied to Fetal Bovine Serum (FBS) for pathogen reduction purposes.
- Electron Beam (E-Beam) irradiation is typically applied to medical devices but is less compatible with serum products.
- Several UV treatment methods are under development for pathogen reduction of platelet apheresis units (starting material for hPL), but are not fully established. Additionally, this process is not feasible for terminal sterilization of final hPL GMP product.

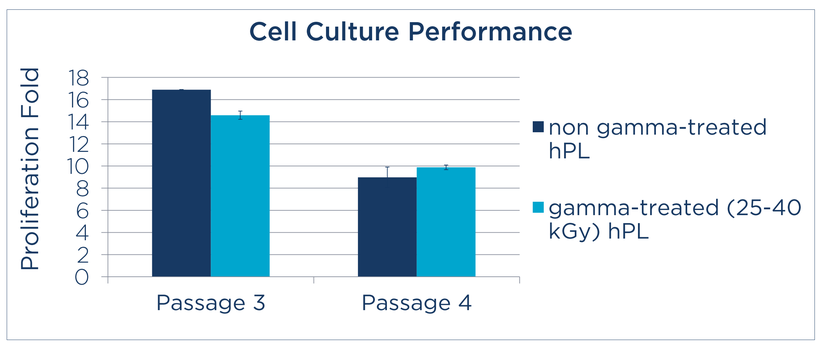
Method	Mechanism	Physical State of Target	Feasible for hPL Terminal Sterilization?	Log_{10} Reduction of Pathogen	Loss in Growth Factors & Cell Culture Performance?
Gamma Irradiation	Ionization radiation	Liquid or frozen	Yes. Method already established and easily available.	>6 log_{10} reduction demonstrated at 25-35 kGy in frozen FBS[5]	≤25% loss at 25-35 kGy (see below)
Electron Beam	Ionization radiation	Liquid or frozen (limited penetration)	Frozen hPL: Difficult Lyophilized hPL: Yes	6 log_{10} reduction requires >55kGy irradiation dose for frozen FBS[4]	Dramatic loss in cell culture performance at 55 kGy (see below)
UVA/Amotosalen – Intercept® (Cerus)	Photochemical crosslinking of nucleic acids	Liquid only	No. Requires removal of Amotosalen and photo by-products	4.5-6.4 for enveloped, 3.5-5 for non-enveloped viruses in platelet units[1]	Non-significant[2]
UVB/Riboflavin – Mirasol® (Terumo)	Photochemical breakage of nucleic acids	Liquid only	Maybe. Extensive on-site method development needed.	2.1-5.9 for enveloped, >5 for non-enveloped viruses in platelet units[1]	Data not available. Increases ROS in treated platelet concentrates[3]
UVC – Theraflex (MacoPharma)	Short-wave UV light blocks transcription	Liquid only	Maybe. Extensive on-site method development needed.	1.4-5.4 for enveloped, >5 for non-enveloped viruses in platelet units[1]	Data not available

(1) Kaiser-Guignard J et al. The clinical and biological impact of new pathogen inactivation technologies on platelet concentrates. *Blood Rev.* 2014 Nov;**28**(6):235-41.
(2) Iudicone P et al. Pathogen-free, plasma-poor platelet lysate and expansion of human mesenchymal stem cells. *J Transl Med.* 2014 Jan **27**;12:28.
(3) Johnson L et al. Treatment of Platelet Concentrates with the Mirasol Pathogen Inactivation System Modulates Platelet Oxidative Stress and NF-κB Activation. *Transfus Med Hemother.* 2015 May;**42**(3):167-73.
(4) Preuss T et al. Comparison of two different methods for inactivation of viruses in serum. *Clin Diagn Lab Immunol.* 1997 Sep;**4**(5):504-8.
(5) Effectiveness of Virus Inactivation by Gamma Irradiation for FBS Product. Published online by Thermo Fisher Scientific.

Effect of Gamma Irradiation on hPL

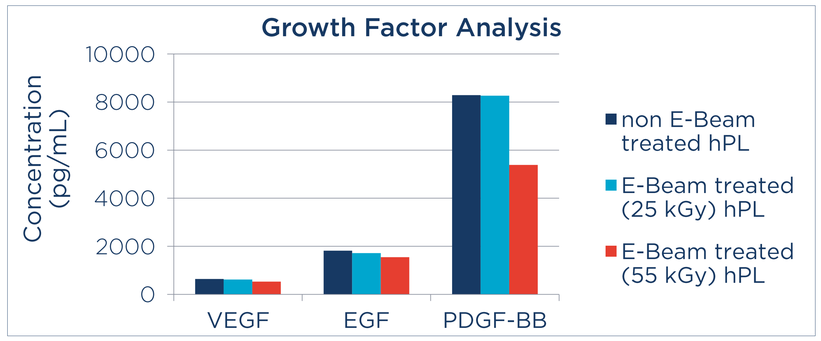


Growth factor concentrations measured using ELISA kits from R&D Systems. Gamma irradiation (at 25-40 kGy) of frozen PLUS™ hPL did not compromise its growth factor content.

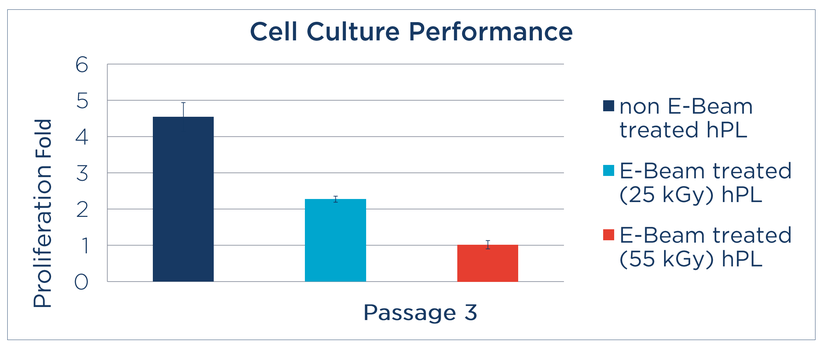


Medium supplemented with 5% (v/v) Gamma irradiated (25-40 kGy) PLUS™ hPL resulted in less than a 25% decrease in human dermal fibroblast proliferation.

Effect of Electron Beam on hPL



Growth factor concentrations measured using ELISA kits from R&D Systems. E-beam irradiation resulted in minimal loss in VEGF and EGF but a dose-dependent loss in PDGF-BB for lyophilized PLUS™ hPL.



Medium supplemented with 5% (v/v) E-beam irradiated (25 kGy) lyophilized PLUS™ hPL resulted in >50% decrease in human fibroblast proliferation. At 55 kGy there was a complete loss in proliferation.

Conclusions and Future Plan

- Gamma irradiation is a preferred pathogen reduction method for hPL.
  - High penetration depth and the greatest pathogen reduction efficiency
  - As a well-established technique, Gamma can be easily adopted to hPL GMP products unlike the UV methods
  - Less damaging effects to hPL performance than E-beam irradiation
- Gamma Irradiation at 25-35 kGy will be an efficient method for complete pathogen reduction[5] and does not significantly compromise hPL performance as a cell culture supplement.
- Future Plan: Finalize the validation of gamma irradiation process and offer PLUS™ hPL as a GMP grade, pathogen inactivated product.