# Establishing Human Platelet Lysate as a Dependable Option for Enabling Cell Production

May 19, 2017 | Brad King | Director of Advanced Laboratory Products

# What questions are we tackling?

- Why are we still using FBS?
- Why try HPL? **Major decision drivers**
- How is HPL produced? **Multiple approaches**
- What are some results using HPL? **3 cases**
- How is HPL regulated? **US and Europe**
- Want more data on HPL?

  ***Dr. Qasim Rafiq (UCL) webinar June 20***

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# Enabling Technologies – Ancillary Materials

**Researchers and manufacturers need tools they can depend on from early stage discovery through pre-clinical development and commercial application**

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# Enabling Technologies – Ancillary Materials

**Dependable options for cell culture media**

**What do we use now?**

**Fetal bovine serum (FBS)**

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# Why are we still using FBS?

## Old habits die hard

- Significant lot-to-lot variability
- Limited supply based on known applications
- Possible animal-derived pathogen transmission
- Immune reaction to bovine proteins
- Increasing cost

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# Why are we still using FBS?

**MSC-Based Product Characterization for Clinical Trials: An FDA Perspective**

**>80% of INDs for MSC therapies included FBS**

*Mendicino M et al (2014). MSC-Based Product Characterization for Clinical Trials: An FDA Perspective, Cell Stem Cell, 14 (2) 141-145*

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# Why are we still using FBS?

## Peak serum: implications of serum supply for cell therapy manufacturing

"Current stocks and production rates of serum suitable for GMP manufacture may only be sufficient to support the production of one blockbuster cell therapy."

"The serum availability bottleneck is not theoretical but already starting to impact the sector, as can be witnessed by the significant rises in the price of GMP-grade serum in recent years."

"Given the decline in both serum processing and its underpinning infrastructure, its role as a key manufacturing component for the future CTI is not tenable."

"At current elevated prices of approximately US$250 per liter ... where 1–3 fetuses are required to produce each liter of serum, it is not cost effective to breed cattle purely for serum production."

*Brindley DA et al (2012). Peak serum: implications of serum for cell therapy manufacturing, regen med, 7(1), 7-13*

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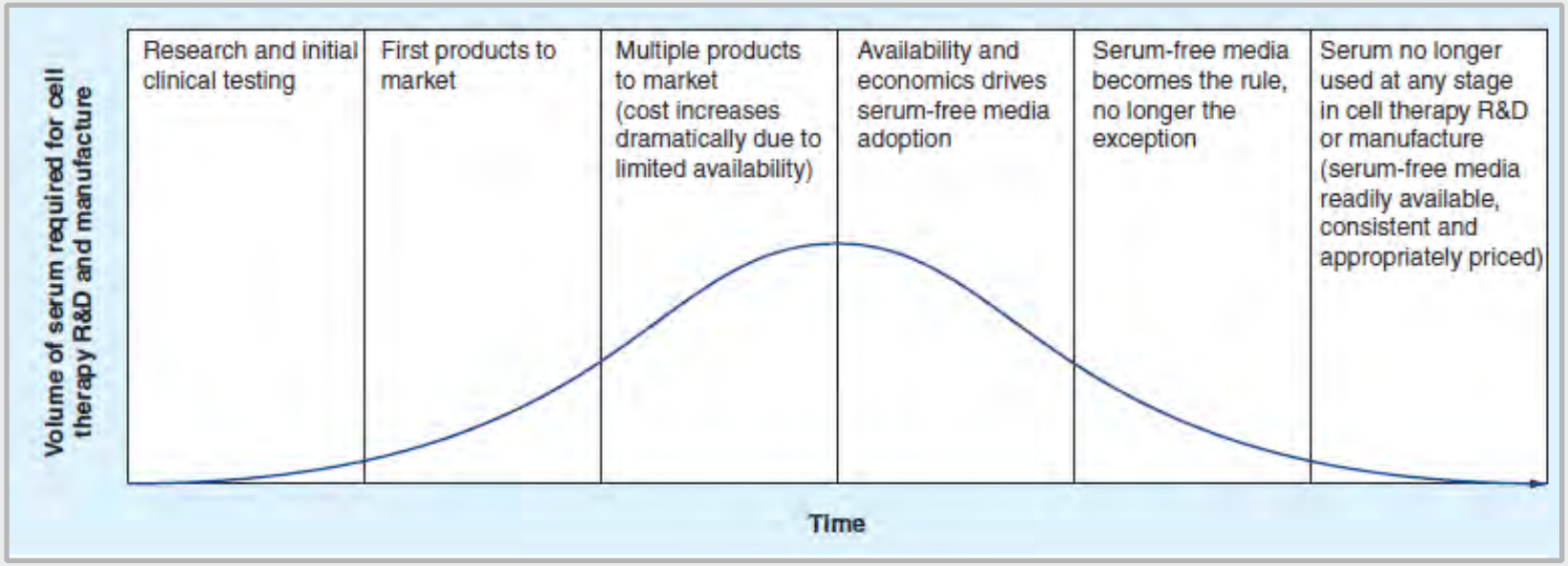
# Why are we still using FBS?

## How will FBS use change when the first CT products are on the market?




*Brindley DA et al (2012). Peak serum: implications of serum for cell therapy manufacturing, regen med, 7(1), 7-13*

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# Is defined media the holy grail?

Chemically defined media might be preferred in theory but…

- Does the benefit justify the cost
- Does it perform well in 3D culture
- Access to recombinant proteins with increasingly complex media

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# Why try HPL?

**HPL is a human derived and ethically sourced supplement to support cells in culture**

**Commercially available since 2010**

**Used in clinical studies around the world**

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# How is HPL produced?

- Starting material is from qualified blood centers
- Volunteer donors that meet screening criteria
- Transfusable units that have been fully tested to regulatory standards
- Provided to HPL manufacturers after expiration

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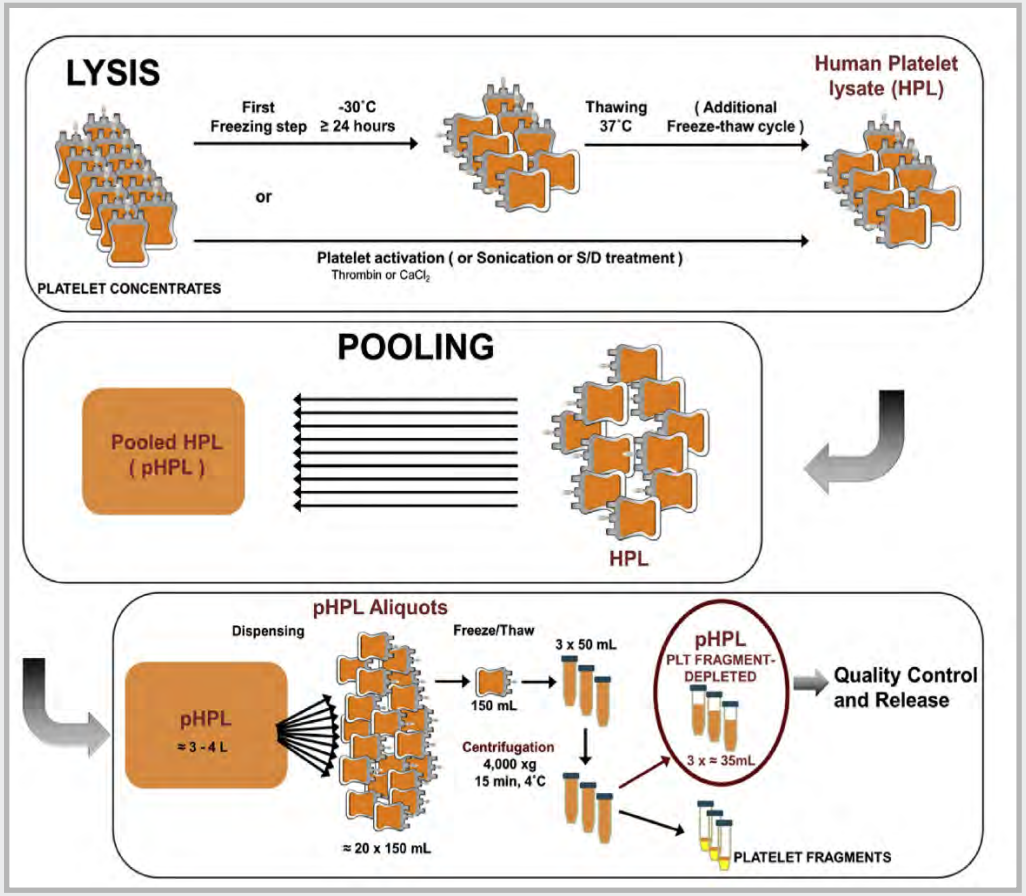
# How is HPL produced?

- Source of platelets
- Pooling – lot size
- Addition of heparin
- Fibrinogen depletion
- Fill volumes
- Final container
- Frozen product
- Lyophilized product




Burnouf T *et al (2016)*. HPL: Replacing FBS as Gold Standard for human cell propagation, Biomaterials 76, 371-387

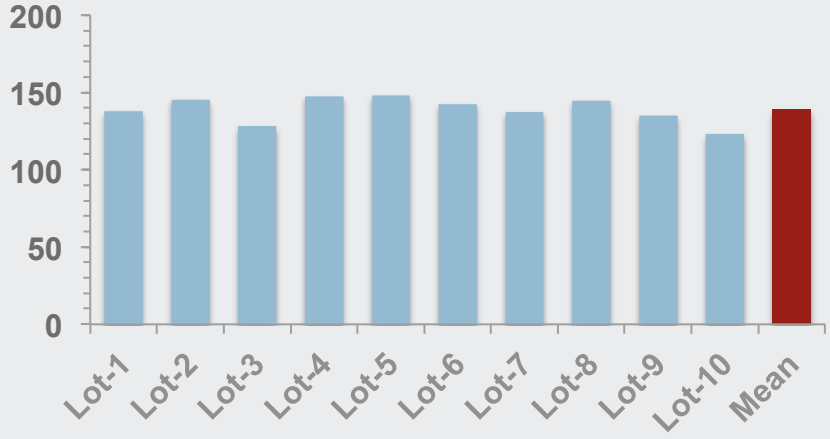
# What is the content of HPL?

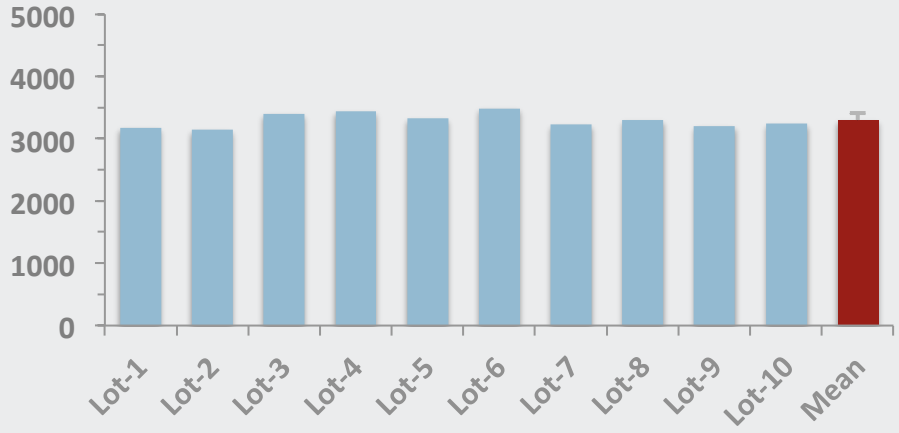
## Representative certificate of analysis for HPL

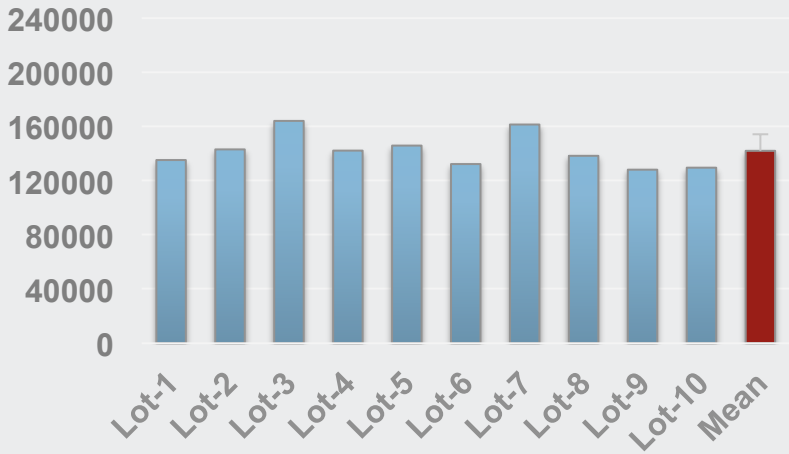
| Test | Specification |
|---|---|
| Endotoxin | < 10 EU/mL |
| Mycoplasma | No Detection |
| Total Protein | 4-6 g/dL |
| Osmolality | 260-340 mOsm/kg |
| pH | 6.8-7.8 |
| Sterility | No growth |
| Cell Culture | Pass |

# What is the content of HPL?

## Lot-to-lot consistency of HPL



# How does HPL composition compare?

## What is the content of defined media?

- Defined by the manufacturer but not necessarily shared with the user

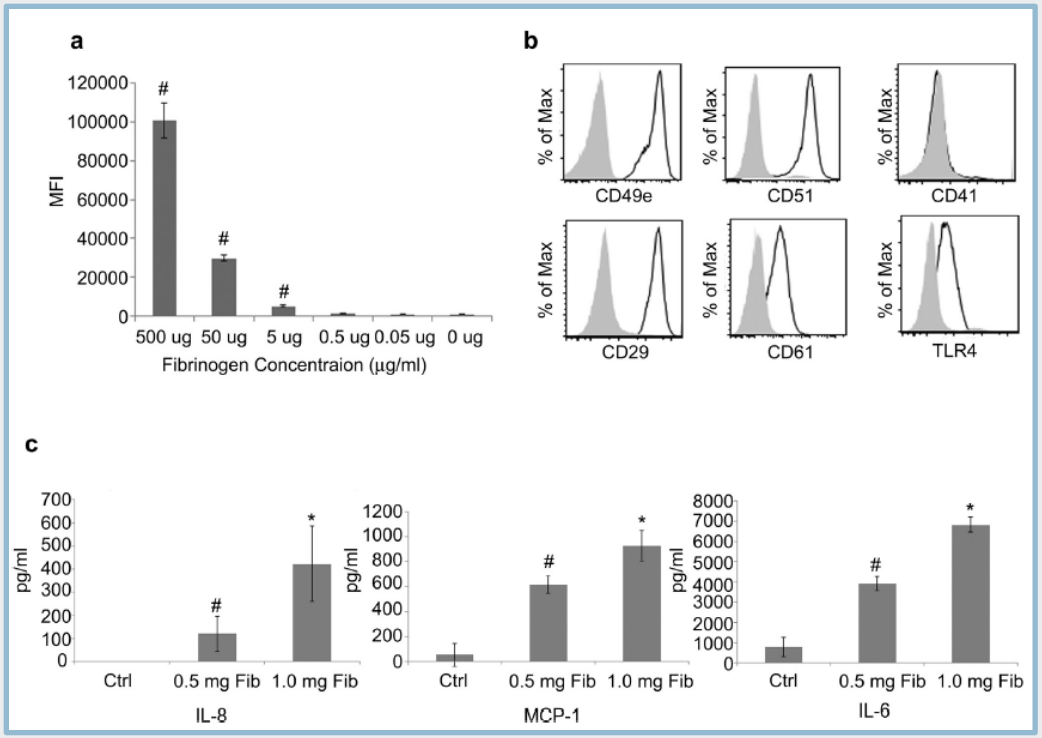
## What is the content of FBS?

**Growth Factor Levels in FBS**

| Growth Factor | Unit | FBS | CH/DX FBS | Dialyzed FBS |
|---|---|---|---|---|
| IGF-1 | ng/mL | 111 | 49.3 | 98.7 |
| TGF-Beta1 | ng/mL | 12.6 | 7.3 | 12.3 |
| FGF-2 | pg/mL | 37.3 | 32.7 | 43.3 |

See product descriptions. All results are the average of three lots. For testing methodology, more information and discussion, including possible effects on cell culture, please see the following Art to Science Newsletter: Art to Science, Vol. 19 No. 1, Art to Science, Vol. 19 No. 3 and Art to Science, Vol. 20 No. 1. These can currently be found at www.thermo.com/hyclone.

# Why is fibrinogen depletion important?




**Short term exposure of MSCs to fibrinogen caused increased expression of pro-inflammatory cytokines**

*Copland (2013). The effect of platelet lysate fibrinogen on the functionality of MSCs in immunotherapy, Biomaterials 34(32), 7840-7850*

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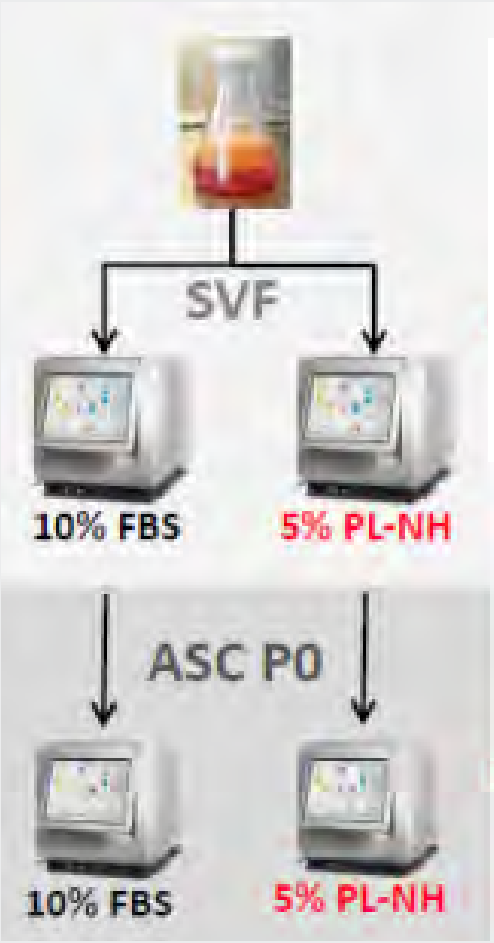
# How is HPL performing?




REGION H Rigshospitalet

A highly specialised hospital in Denmark

ASC P1

| N = 3 | ASC P0 seeded | Days in culture | ASC P1 harvested | PD |
|---|---|---|---|---|
| FBS | 2.10E+07 | 21 | 1.19E+08 | 2.45 |
| PL-NH | 2.10E+07 | 6 | 7.89E+08 | 5.23 |

*Haack-Sorensen, M. et. al, Manuscript in preparation.*

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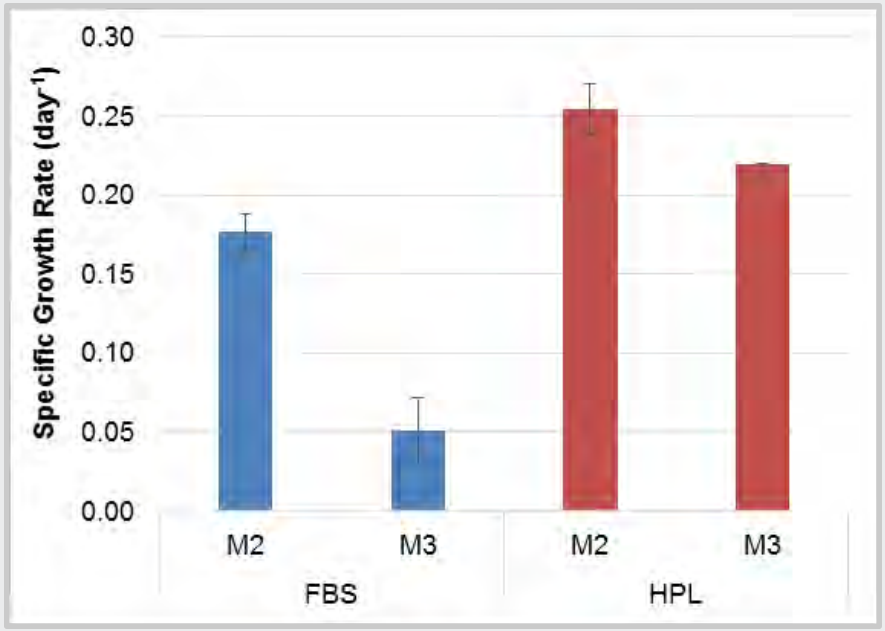
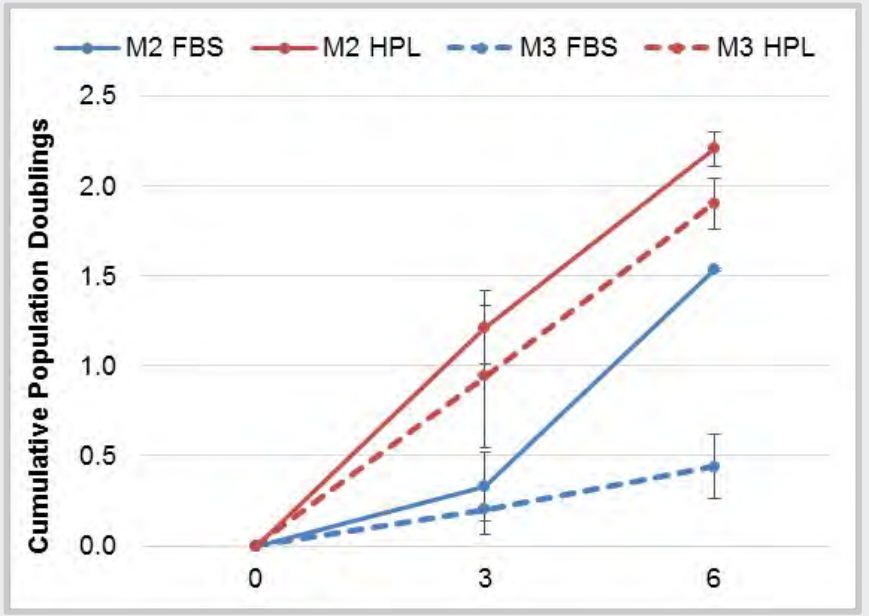
# How is HPL performing?




Loughborough University

*Heathman TRJ et al (2015). Scalability and process transfer of mesenchymal stromal cell production from monolayer to microcarrier culture using human platelet lysate, Cytotherapy 18(4), 523-535*

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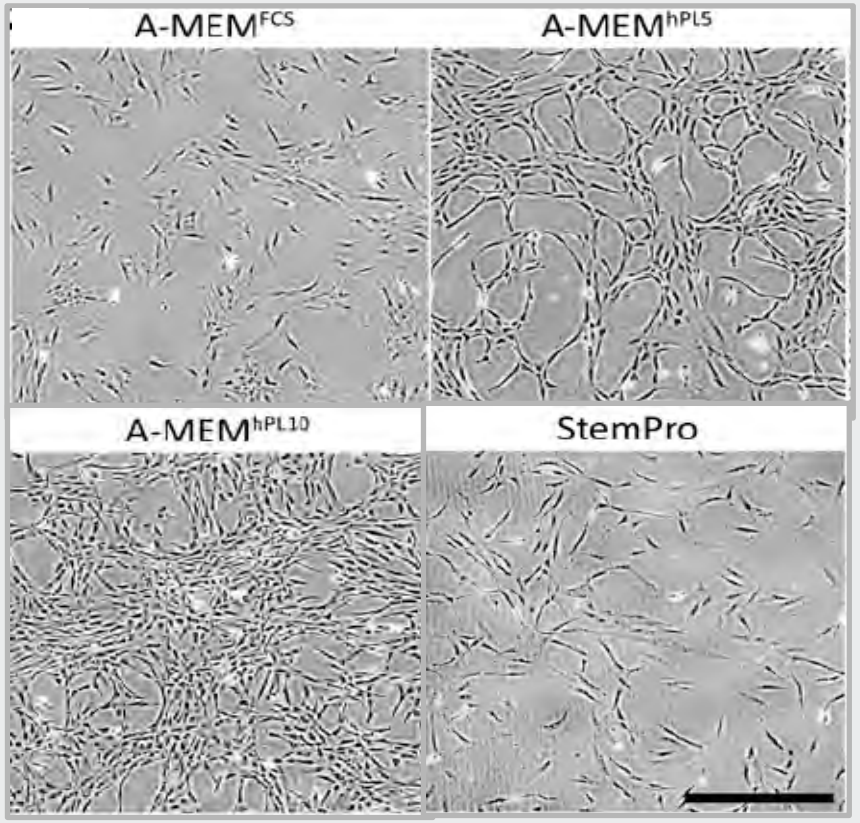


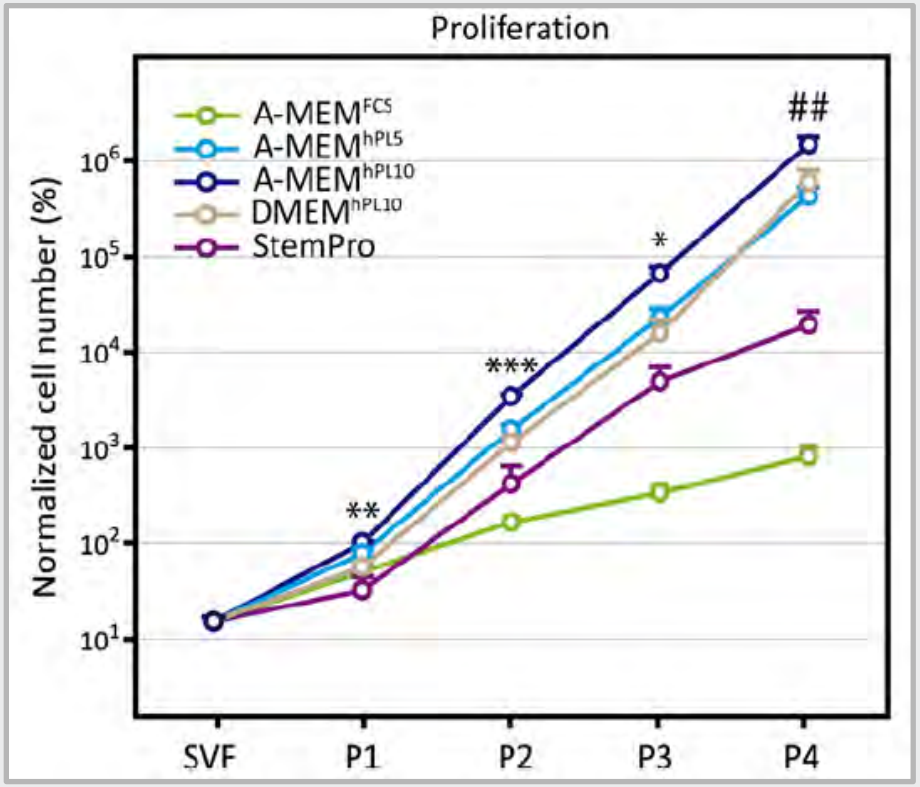


*Riis et al (2016). Comparative Analysis of Media and Supplements on Isolation and Expansion of ASCs, Stem Cells Trans Med;5:1-11*

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# How is HPL performing?

**Avoid the trap of comparing products on a price per liter basis**

- Time to cell dose
- Total number of cells
- Time in manufacturing environment
- Impact on performance

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# How is HPL regulated?

The FDA does not regulate ancillary materials

… but they do regulate the products they touch

In Europe, HPL is a raw material and is typically required to meet pharmacopeial standards …

# How is HPL regulated?

## European Pharmacopeia General Chapter 5.2.12

### Raw Materials of Biological Origin for the Production of Cell-Based and Gene Therapy Medicinal Products

**Because of the inherent risk of transmitting infectious agents from pooled plasma, pooled sera or other derivatives from pooled, allogeneic 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.**

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# How is HPL regulated?

## Approaches to pathogen reduction

- Pasteurization
- Dry heat/lyophilization
- Low pH
- Irradiation
- Solvent-detergent
- Nanofiltration
- Photochemical processes

## How much is enough?

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# What is the future for HPL?

- Scale and long-term platelet supply
- Approaches to pathogen reduction
- Component in xeno-free media
- Fractionation and other treatments

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# Want more data on HPL?

**LIVE Webinar** hosted by RegMedNet in association with Cook Regentec

***Why are we still using FBS in our processes?***

**Speaker:** Dr. Qasim Rafiq,
University of College London
June 20 | 11:00 | 16:00

Connect with Cook Regentec to receive an invite:
CookRegentec.com/connect

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# Thank you.

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