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Opinion

Production and Quality Requirements of Human Platelet Lysate: A Position Statement from the Working Party on Cellular Therapies of the International Society of Blood Transfusion

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Human platelet lysate (HPL), rich in growth factors, is an efficient alternative supplement to fetal bovine serum (FBS) for *ex vivo* propagation of stromal cell-based medicinal products. Since 2014, HPL has been a focus of the Working Party for Cellular Therapies of the International Society of Blood Transfusion (ISBT). Currently, as several Good Manufacturing Practice (GMP)-compliant manufacturing protocols exist, an international consensus defining the optimal modes of industrial production, product specification, pathogen safety, and release criteria of this ancillary material (AM) is needed. This opinion article by the ISBT Working Party summarizes the current knowledge on HPL production and proposes recommendations on manufacturing and quality management in line with current technological innovations and regulations of biological products and advanced therapy medicinal products.

Highlights

Human platelet lysate (HPL) is an effective novel growth medium supplement for xenofree *ex vivo* propagation of human cells for cell therapy and regenerative medicine.

Consensus is needed to ensure the quality and safety of HPL supplements regarding the source of platelet concentrates, donor variability, manufacturing processes, and minimum release criteria.

It is critical to guarantee HPL pathogen safety by implementing measures including screening blood donors, pathogen testing of platelet concentrates, and, for large pools, implementing dedicated virus reduction treatments.

International consensus between the various stakeholders (blood establishments, the biotechnology industry, and regulators) seems close to delineating the required quality and safety criteria.

The development of functional correlates for the various cell types supported by HPL is needed.

Definition and Use of HPL

HPL is a cell-free, protein and growth factor-rich biological material that is produced mostly from expired clinical-grade human **platelet concentrates (PCs)** (see Glossary) initially intended for transfusion. Numerous studies have now proved that HPL is, in particular, an excellent clinical-grade supplement of growth media used for the phases of *in vitro* culture and expansion of therapeutic cell-based medicinal products [1–3]. HPL is, therefore, emerging as an efficient substitute for **FBS** as a **xenofree** growth medium supplement; its human origin alleviates immunological and infectious safety concerns associated with the use of materials from bovine origin. It also provides a currently suitable **GMP**-compliant alternative to 'chemically defined' media when those are not efficient for the culture of primary cells [1,4,5]. When compared with FBS for the expansion of **mesenchymal stromal cells (MSCs)** from various tissues, use of HPL generally results in better cell expansion and shorter doubling time as well as maintenance of cellular immunophenotype, immunosuppressive function, and differentiation capacity. Moreover, these expanded cells did not stimulate tumorigenicity *in vivo* [1]. Considering the role that HPL is going to play in the biotechnology and cell therapy industry, an impetus to achieve consensus on quality and safety criteria is urgently needed.

Human PC as Source Material for HPL Production

HPL is produced from PC initially intended for transfusion purposes (Figure 1). PC can be prepared from 200–500 ml anticoagulated **whole-blood donations**, as a byproduct of the preparation of red blood cell concentrates and plasma, or using a dedicated automatic **platelet** collection procedure called **plateletpheresis**. In the PC (approximately 150–300 ml) platelets are enriched four- or fivefold compared with the physiological level in the blood circulation. To reach the required amount of platelets suitable for transfusion, and a sufficient hemostatic effect in patients, the '**buffy coat**' units of four to six whole-blood-derived platelet donations are pooled to prepare a therapeutic dose for adult patients. For single-donor PC from plateletpheresis, no **pooling** is needed. The platelets can be suspended in plasma or in a mixture of plasma and platelet additive solution (PAS). According to the Council of Europe *Guide to the Preparation, Use and Quality Assurance of Blood Components* [6], the minimum platelet content should be 2×10^{11} with less than 1×10^{6} residual leukocytes per therapeutic unit. The pH should be more than 6.4 at the end of the shelf life. PC must be tested negative

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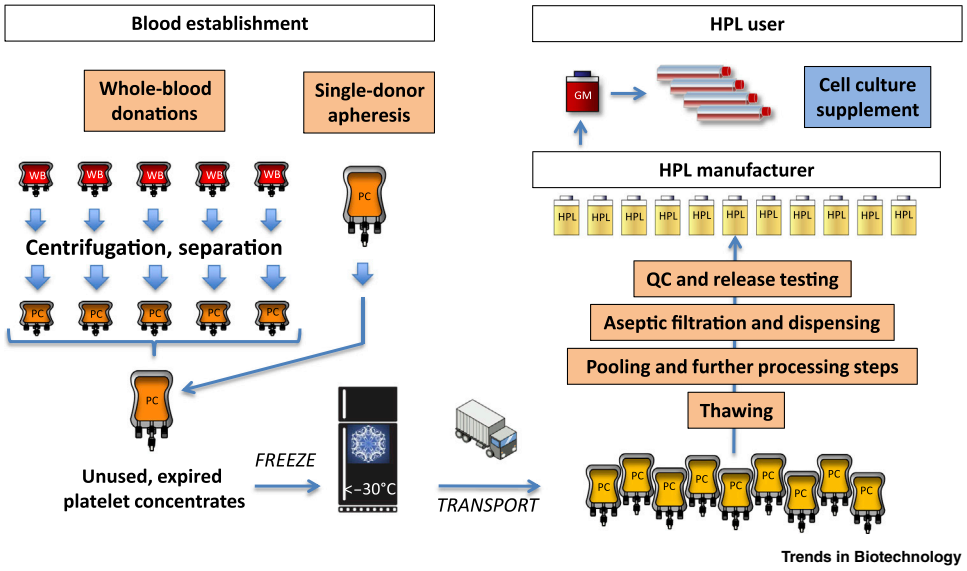

Figure 1. Overall Production Scheme for Human Platelet Lysate (HPL).
Platelet concentrates (PCs) are prepared by licensed blood establishments from whole blood (WB) or are collected by apheresis. When reaching the expiry date, the unused PCs are frozen and transported to the HPL production facility. The PCs are thawed, pooled, processed, and dispensed into the final HPL. The HPL is used as supplement in cell growth medium (GM).

for microbiological contaminations such as bacteria and fungi. Table 1 summarizes the set of quality controls for PC as raw materials to produce HPL recommended by the Working Party.

Only regular, traceable, clinical-grade PC produced by licensed **blood establishments** should be used as source material for HPL. Acceptable variations are the following: (i) PC may be derived from apheresis, platelet-rich plasma, or pooled buffy coats; (ii) PC may contain 100% plasma or 30–40% plasma and 60–70% PAS; and (iii) PC may be gamma irradiated (30 Gy) or may be pathogen reduced by techniques licensed for this product (see below). As plasma of female donors may contain antibodies directed against human leukocyte antigens (HLAs) and human neutrophil antigens (HNAs) from previous pregnancies, using PC only from male donors or negatively tested female donors may be considered to prevent any possible transmission to the recipients of expanded cells. It remains, however, unknown whether such a risk does exist.

The shelf life of PC, which is guided by the need to limit the risk of bacterial growth, is 3–7 days at 22 ± 2°C, depending on legislation. This short shelf life, associated with the need to ensure a sufficient inventory of platelets for transfusion medicine, leads to the fact that, at least in some countries, up to 10–20% of produced PC cannot be transfused and are thus discarded [7]. PC not transfused within this time frame of their collection can be frozen and used as raw material to prepare HPL without noticeable impact on subsequent cell propagation [8,9]. The committee believes that freezing of PC within 7 days after collection for further manufacture into HPL is readily feasible by blood establishments and contributes to limiting the risk of bacterial growth. However, the maximum period time that PC can be used after expiry to prepare an efficient HPL for cell expansion is unknown.

Methods for HPL Manufacture and Pool Size to Ensure Consistency

Various protocols for **platelet lysate** generation and further processing of HPL are available from the literature and a comprehensive overview exposed in a recent review [1]. The pooling of a sufficient number of PC is intended to counterbalance possibly fluctuant growth factor concentrations in different PC donations. In a recently published international survey [2], all participating centers stated

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Table 1. Quality Controls for PCs as Starting Materials for HPL Production[a,b]

	Parameter	Specification range	Frequency of control
Infectious disease markers	Anti-HIV1/2, HBV surface antigen, anti-HCV, HIV NAT, HBV NAT, HCV NAT, syphilis, and/or others in compliance with national requirements	Negative by approved screening test	All units
Sterility	Bacteria and fungi	Negative by approved screening test	All units
Biochemical analysis	pH	>6.4 at the end of shelf life	As determined by SPC
Volume		>40 ml per 0.6×10^{11} platelets	As determined by SPC
Cell content	Platelets	$>2 \times 10^{11}$/unit	As determined by SPC
	Residual leukocytes	$<1 \times 10^{6}$/unit	As determined by SPC

[a]Abbreviations: NAT, nucleic acid amplification test; SPC, statistical process control.
[b]Modified from [6].

to prefer pooling to avoid previously reported donor variations [10,11]. However, even for GMP-grade manufacture, the number of pooled lysates was reported to be highly variable (from 4 to 125 PC units) [2]. Other published studies have used a pool size of 10–15 PC, corresponding to 40–50 individual donations [12,13], to provide a sufficient level of standardization. Besides, as described below, due to the risk of transmission of pathogens the number of pooled blood products should be limited, as recommended by the European Pharmacopoeia (Chapter 5.2.12) [14] unless HPL, and/or PC, undergoes **pathogen inactivation** or reduction. Also, in the US Pharmacopeia (USP) (Chapter <1043>) [15], a qualification program for **AMs** as platelet lysate and a risk-based approach is suggested (Chapter <1046>) [16].

A crucial step in HPL production is the lysis or activation of platelets to release stored active substances such as growth factors, cytokines, and chemokines into the plasma or PAS/plasma. The efficiency of this procedure can have an essential impact on HPL composition. A previous literature search on HPL for research [1] revealed that the most frequent procedure is freezing–thawing (74%), followed by platelet activation by addition of thrombin or other agonists (13%), platelet sonication (8%), solvent/detergent (S/D) treatment of PC (2%), and others not defined (3%). As summarized from an international questionnaire [2], seven centers producing GMP-compatible HPL specified several freeze–thaw cycles (−20 to −80°C and 37°C) to induce platelet lysis. The Working Party recommends performing three to five cycles of freeze–thaw, as a recent study using −70°C/+37°C has shown a maximum release of growth factors [17]. Most producers implemented a final centrifugation step to pelletize and remove platelet fragments.

The typical protein and growth factor composition of HPL is indicated in Table 2. The main factors influencing the protein composition are: (i) the use of PAS during the preparation of PC; and (ii) the implementation of serum conversion during HPL production. The dilution of the plasma compartment by PAS proportionally decreases the total plasma protein content, but not the concentration of platelet-derived factors. Serum conversion, achieved by inducing clotting of the lysate generally by the addition of calcium chloride, leads to depletion of fibrinogen and coagulation factors. It also enables the avoidance of heparin that is otherwise needed to prevent growth medium gelation during cell cultures. Several studies have reported that 5–10% (v/v) HPL, regardless of variations in production methods and protein content, performs better than 5–15% FBS in promoting MSC expansion [1]. Therefore, the Working Party does not recommend one production method of HPL over another.

Glossary

Ancillary material (AM): components, reagents, or materials (e.g., HPL) used for cell expansion but not intended to be part of the final cell product.
Blood establishment: a licensed facility responsible for any aspects of the collection and testing of human blood or blood components and their processing, storage, and distribution when intended for transfusion or further manufacture.
Buffy coat: a fraction of centrifuged anticoagulated whole blood enriched in platelets and leukocytes (white blood cells).
Downstream processing: all production steps implemented to formulate the platelet lysate into a HPL product meeting intended quality specifications for clinically related application of the cell product.
Fetal bovine serum (FBS): a medium supplement used as a source of nutrients and growth factors for cell culture. It is collected from the blood of calves after clotting to generate serum.
Good Manufacturing Practice (GMP): a legally binding system, part of quality assurance, for ensuring that HPL products are consistently produced and controlled according to quality standards approved by regulatory authorities.
Mesenchymal stromal cells (MSCs): stromal cells with the potential to differentiate into at least three lineages of cell types. MSCs can be cultured in HPL-supplemented medium.
Pathogen inactivation: a dedicated and validated treatment specifically intended to destroy the infectivity of pathogens (e.g., viruses, bacteria, parasites) that may be present in starting PCs or in HPL.
Pathogen removal: a dedicated and validated production treatment specifically intended to remove bacteria or viruses that may be present in HPL.
Platelet: an anucleate blood cell of 2–3 μm and a lifespan of 7–10 days in the circulation that is instrumental in the control of hemorrhage. Platelets contain numerous factors (growth factors, cytokines, coagulation factors,

When concerns exist about the use of animal-derived heparin [18] to counterbalance the presence of coagulant fibrinogen, serum-converted HPL should be preferred.

Quality Control Parameters for HPL

Standardization of quality control and **release testing** of HPL has to consider regulatory requirements for raw materials or AMs as well as product specifications defined by the manufacturer. USP 37 describes in chapters <1043> [15] and <1046> [16] the need for identification, selection, and suitability for use, characterization, vendor qualification, quality assurance, and control. For the risk classification of each AM, the source and processes employed in its manufacture should be taken into account: 'Whenever available, AMs that are approved or licensed therapeutic products are preferable because they are well-characterized with an established toxicological profile and are manufactured according to controlled and documented procedures'. Tiers of sample risk categories are provided as a guide. For example, human serum albumin is evaluated at low risk as a drug for injection whereas FBS, as an animal-derived substance, is at high risk [15]. For the GMP-compliant production of HPL, it is necessary to perform adequate qualification or risk reduction activities.

In Vitro Assays

The first step of the HPL workflow is the evaluation and qualification of the blood donors by a questionnaire and physical examination before drawing blood. Donors with defined pre-existing diseases or with a potential risk for the transmission of infectious diseases must be excluded. As required by the national and international competent authorities, donations should be tested for the absence of defined infectious diseases markers, analysis of ABO and RhD blood groups, and irregular antibodies. Sterility testing of PCs derived from whole-blood donations or apheresis must be negative. After lysis of platelets and further technical steps as described above, the final HPL product has to be evaluated, usually by in-house quality control to meet release criteria. General quality requirements of raw materials are defined in Chapter 5.2.12 of the European Pharmacopoeia (9.0) [14]. Testing for microbial sterility should include the detection of aerobic and anaerobic bacteria, yeast, and fungi. Several test methods are available: point-of issue tests as PCR assays, flow cytometry, or immunoassays as well the most commonly used BACT/ALERT® 3D system from bioMérieux. Bacterial endotoxins are analyzed by the Limulus Amebocyte Lysate (LAL) endotoxin test and should be below the limit defined for the raw material. *Mycoplasma* represents a large group of microorganisms and is a consistent problem for cell culture. Negative testing in HPL is required as *Mycoplasma* may also be transmitted from humans. Biochemical analyses should include at least testing for osmolality, pH, and total protein as required [16]. As HPL is a plasma-containing product, the analysis of isoagglutinin titers is recommended when cells such as endothelial colony-forming progenitor cells (ECFCs) expressing blood group AB antigens [19] are cultured, although previous ECFC studies did not consider HPL isoagglutinin [20,21]. The acceptable thresholds should be defined, depending on cell proliferation tests and the application of a risk-based approach.

In Vitro Cellular Assays

As HPL is a complex mixture of various not sufficiently defined substances, for identification and characterization a so-called performance testing is required [15]. Such performance testing is also important to limit lot-to-lot variability and because no simple quality control test exists. It should be performed using a reference HPL batch and the same reference cells.

Table 3 provides an example of a set of quality specifications for HPL suggested by the Working Party. The ranges and values indicated for proteins, including growth factors, may be product specific, being dependent on the PC source and the HPL production process.

HPL Pathogen Safety

Several types of donor-blood-derived pathogens can contaminate freshly collected PC: bacteria (e.g., spirochetes, the agent of syphilis), parasites (e.g., *Babesia microti*, *Plasmodium*, *Leishmania*, *Trypanosoma cruzi*), or viruses. This can be a major cause of transfusion-transmitted infections

etc.) essentially contributing to tissue repair and regeneration.
Platelet concentrate (PC): a therapeutic blood product, collected in blood establishments from whole-blood donations or by apheresis, used to treat bleeding disorders associated with platelet depletion or dysfunction occurring in some diseases or resulting from medical treatments.
Platelet lysate: a complex protein fluid rich in various nutrients and growth factors, which is obtained by lysis or activation (degranulation) of PCs.
Plateletpheresis: automatic dedicated collection procedure (also called thrombocytapheresis) assisted by an apheresis machine whereby blood is taken from a donor and separated by physical means to recover a concentrate of platelets that is suspended in 100% plasma or a mixture of plasma and PAS.
Pooling: a production step of mixing multiple donations or intermediates.
Prions: transmissible infectious protein particles, supposed to be able to self-replicate and responsible for diseases of the nervous system called TSEs, such as mad-cow disease and vCJD in humans.
Release testing: analysis of defined parameters using validated methods and verifying that the final product's manufacture meets approved and documented protocols and pre-established quality specifications.
Whole-blood donation: a procedure whereby a single donation of blood is collected into a plastic-bag system containing an anticoagulant and a red blood cell-stabilizing solution.
Xenofree: terminology used to define that all components in the culture medium derived are from the same species as the cells (i.e., for human cells this means free of animal-derived components).

Table 2. Example of the Protein Content of HPLs, Depending on Characteristics of the PC and Mode of Platelet Lysis[a]

	Component	(A) PC formulated in 100% plasma		(B) PC formulated in PAS/plasma	
Mode of preparation of the platelet lysate		(A1) Freeze–thaw	(A2) Thrombin/$CaCl_2$ treatment	(B1) Freeze–thaw	(B2) Thrombin/$CaCl_2$ treatment
Plasma compartment	Total proteins (g/dl)	6.5–8.5	6.0–8.0	The plasma protein composition is qualitatively the same as in (A1), but with a dilution factor equivalent to that of plasma in PAS	The plasma protein composition is qualitatively the same as in (B1), but with a dilution factor equivalent to that of plasma in PAS
	Albumin (g/dl)	3.5–5.5	3.5–4.0		
	IgG (g/dl)	0.8–1.2	0.8–1.2		
	IA (g/dl)	0.07–0.13	0.07–0.13		
	IM (g/dl)	0.03–0.06	0.03–0.06		
	Fibrinogen (g/dl)	0.2–0.4	<0.1		
	IGF-1 (ng/ml)	50–200	50–200		
Platelet compartment	PDGF-AB (ng/ml)	50–300	The growth factor composition and content is expected to be slightly less than in (A1) due to incomplete lysis and entrapment of growth factors in the fibrin clot	The growth factor composition and concentration range is the same as in (A1)	The growth factor composition and content is expected to be slightly less than in (B1) due to incomplete lysis and entrapment of growth factors in the fibrin clot
	PDGF-AA (ng/ml)	10–30			
	PDGF-BB (ng/ml)	1–10			
	TGF-β1 (ng/ml)	50–300			
	TGF-β2 (ng/ml)	~0.5			
	BDNF (ng/ml)	10–50			
	VEGF (ng/ml)	5–10			
	b-FGF (ng/ml)	1–5			
	EGF (ng/ml)	0.5–10			
	HGF (ng/ml)	0.1–2			

[a]Adapted from [1,3].

(TTIs), as the storage of PC at 22 ± 2°C for up to 5 or 7 days supports bacterial growth [22]. The primary origin of bacteria in PC is the skin microflora at the site of venipuncture. Measures to prevent bacterial transmission by PC include donor selection, careful skin disinfection, diversion of the initial volume of blood collected into a discarded pouch, bacterial testing, and pathogen inactivation [22].

The risks of TTI associated with HPL, compared with PC for transfusion, is mitigated by the fact that several PC freeze–thaw cycles implemented for platelet lysis contribute to the destruction of pathogens such as parasites. In addition, the implementation of 0.2 μm sterile filtration(s) during HPL manufacture removes bacteria and parasites. Control of endotoxins in the HPL pool or final product provides an additional safety guard to detect any upstream bacterial contamination. Of course, these measures have no impact on virus safety. Viruses are therefore the primary pathogens of concern with regard to HPL safety. Human blood products can transmit viral infectious agents, including lipid-enveloped viruses [HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and various emerging viruses: e.g., West Nile virus (WNV), dengue virus (DENV), Zika virus (ZIKV)] and nonenveloped viruses [hepatitis A virus (HAV), parvovirus B19, and hepatitis E virus (HEV)] [23–25]. Historical perspectives on the clinical use of industrial plasma-derived coagulation factors indicate that pooling increases the risk of transmission of viruses to recipients. Countermeasures to gradually build up the current virus safety of pooled plasma products have relied on: (i) strict screening of blood/plasma donors; (ii) virus testing of blood/plasma donations at individual and at manufacturing pool levels; and, most importantly, (iii) introducing validated, robust pathogen inactivation and **pathogen removal** steps during

Table 3. Example of a Set of Quality Specifications for HPL[a]

	Parameter	Specification range	Method of testing	Frequency of testing
Pooling	Number of donations	10–16	–	–
Sterility	Bacteria and fungi Endotoxin *Mycoplasma*	Negative <0.5 EU/ml Negative	Automated microbial detection system, PCR, or ELISA LAL endotoxin tests [53] Culture method [54]	All batches
Biochemical analysis	Osmolality pH Total protein	According to the range of standard values for human blood	Osmometer pH-meter Biuret protein assay	All batches (specification for 100% plasma HPL) [14]
Immunology	Isoagglutinins	To be validated	IAT	All batches used for culture of ECFCs
Potency and functionality	Platelet-derived growth factors according to [1] PDGF-AB TGF-β1	 >50 ng/ml >50 ng/ml	 ELISA ELISA	Randomly
	Performance testing	Expected rate and amount of cellular proliferation [15]		Randomly, comparison of cell proliferation rate supported by a standard batch with a new batch using reference cell types intended to be cultured with HPL

[a]Abbreviations: LAL, *Limulus* amebocyte lysate; EU, endotoxin unit (1 international unit of bacterial endotoxin according to [53]); IAT, indirect antiglobulin test.

the production process [23,26] (Figure 2). Combining effective complementary virus inactivation and removal treatments is the best tool to provide an optimal margin of safety against a range of pathogenic plasma-borne viruses [27,28]. Besides, full traceability between individual donations and final blood products is essential by allowing the tracing back of any quality or safety problems, including infectious risks, and the taking of measures needed to protect both the blood donors and the recipients of the expanded cells.

Based on historical perspectives and current knowledge about the main blood-borne viruses, the spectrum of measures recommended to diminish the infectious risks of pooled blood products are listed in Table 4. Applying similar measures will be vital for HPL as it will be used for human cell propagation, with broader categories of patients in the regenerative medicine field exposed to blood-derived products than today. The European Pharmacopoeia recommends a limitation of the number of donations pooled, when there are no virus inactivation or removal steps, but does not give a specific recommendation for the pool size [14]. By contrast, for instance, German regulations by the Paul Ehrlich Institute (PEI) specify the restriction to 16 individual donations when no virus inactivation treatment is applied [29]. One virus inactivation approach is to use PC subjected to a 'pathogen reduction' treatment. PC 'pathogen reduced' by psoralen/UVA [30–32] or by short-wave UV light [33] have been found suitable to prepare HPLs for the propagation of various types of human MSCs. A second

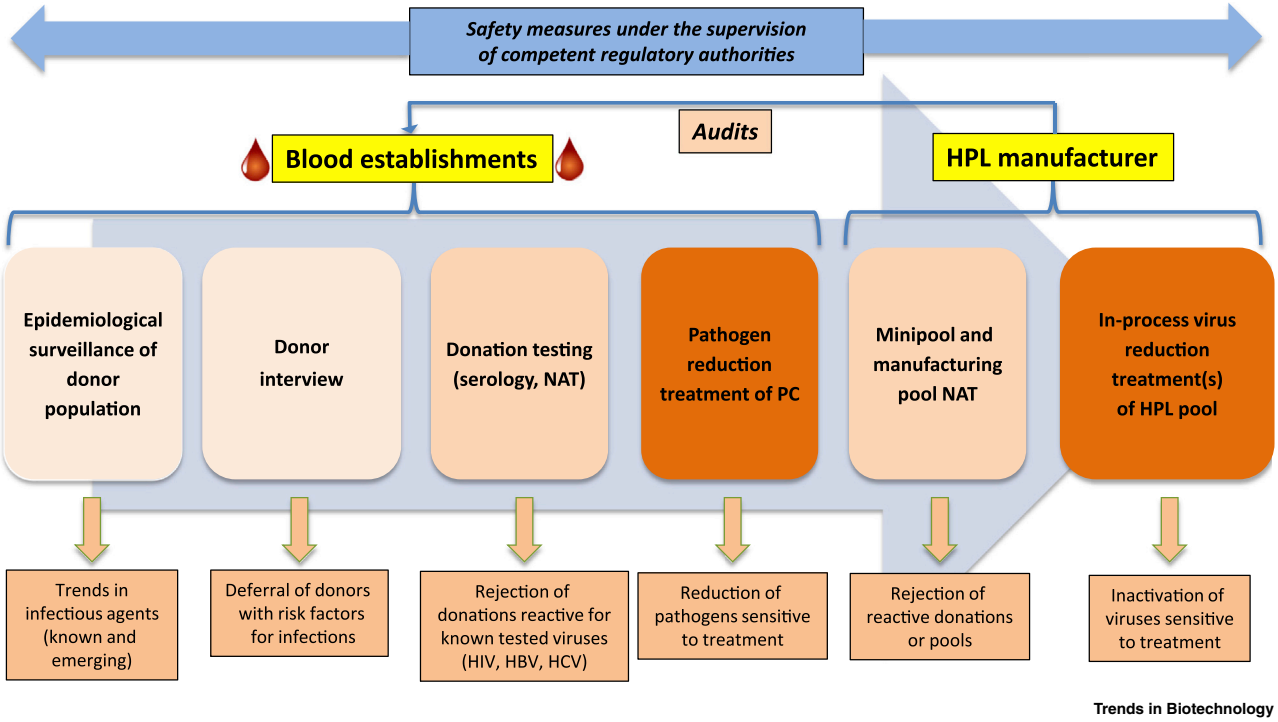

Figure 2. Safety Measures Contributing to the Pathogen Safety of Human Platelet Lysate (HPL).
As for any blood product, the pathogen safety of HPL relies on the complementarity of a set of specific measures, under the control of the competent regulatory authorities, to ensure the optimal safety of the donations (epidemiological surveillance and donor screening) and the implementation of dedicated pathogen testing and pathogen (especially virus) inactivation and removal procedures. NAT, nucleic acid amplification test.

approach comprises implementing a dedicated HPL virus inactivation treatment during **downstream processing**. S/D treatment, which effectively inactivates lipid-enveloped viruses, can be applied without affecting HPL's capacity to expand adipose-tissue- [34] and bone-marrow- [35] derived MSCs. Gamma irradiation of HPL is a recently proposed procedure that provides efficient inactivation of a broad range of viruses without affecting the capability of HPL supplement for MSC expansion [36]. As viral reduction treatments inherently have limitations in the extent or range of virus inactivation, combining approaches, such as psoralen/UVA and S/D, may provide an additional margin of safety needed for industrial-scale HPL pools, as shown recently [37]. Another means is to test the starting HPL manufacturing pool by NAT, as done in the plasma fractionation industry, to verify either the absence of known virus markers, or by immunological assays to determine the presence of neutralizing antibodies to infectious viruses.

In conclusion, the Working Party suggests that applying at least one step of virus inactivation should be a mandatory trend for pooled HPL used for the propagation of therapeutic human cells when the pool size is above 16 individual donations, in line with the recommendations of the PEI [29]. However, the maximum pool size acceptable should be defined based on risk assessment taking into account epidemiology, donor screening, and virus testing strategies. The implementation of a dedicated virus reduction step can allow the processing of larger pool size. As a possible reference, the maximum allowed pool size of industrial S/D-treated plasma for transfusion (a therapeutic product that, like HPL, is not subjected to fractionation steps) varies on legislation. It was set at 60 l in France, 200 l in Germany and South Africa, 380 l in Austria, and up to 650 l in the USA [38]. The need to combine two 'orthogonal' virus inactivation steps may depend on a risk assessment analysis taking into consideration the extent of virus testing done on individual PC donations or manufacturing pool, the HPL pool size, and the extent of pathogen removal or inactivation achieved by measures in place.

Table 4. Current Building Blocks and Additional Virus Safety Steps Considered for Pooled HPL (Based on Experience and Regulations for Industrial Plasma Products)

	Epidemiological surveillance	Repeat donors	Donor screening	Donation testing	Pathogen reduction treatment of PC[a]	Manufacturing/ minipool testing	Dedicated virus reduction step	Final product testing
Status	Already in place for preparing PCs for transfusion in countries applying GMP principles [55]						Under development	
Entity responsible	Blood establishment [55]					HPL producer		
Audits	To be performed by HPL producer [23]							
Virus target	Known (HIV, HBV, HCV) and emerging (e.g., ZKV, WNV, DENV, HEV) viruses			HIV, HBV, HCV	All viruses[b]	HIV, HBV, HCV (HAV, B19)[b]	All viruses[c]	HIV, HBV, HCV markers
Objective	To know the prevalence and incidence, and their respective trends, of infectious markers relevant to the safety of blood components [55]	To build up medical health records of donors	To check that the donor is in good health, with no risk factors for infections	To use only donations nonreactive for anti-HIV, anti-HCV, HBsAg, and nucleic acid testing (HIV, HBV, HCV)[d]	To inactivate pathogens in PC	To ensure that the HPL manufacturing pool is nonreactive for markers of known tested viruses	To inactivate a broad range of viruses in HPL; two methods described: S/D and gamma irradiation	To reconfirm that PC and HPL batch were nonreactive for virus markers

[a]Some pathogen reduction treatments are licensed in most HDI countries.
[b]Potentially other viruses as needed.
[c]Technologies in use may have limits on their capacity to inactivate some viruses.
[d]NAT testing may be mandatory for other viruses (e.g., ZIKV, WNV) in some legislation.

HPL Prion Safety

Prions are very resistant, unconventional infectious misfolded proteins mostly present in the central nervous system and responsible for transmissible spongiform encephalopathies (TSEs). TSEs include bovine spongiform encephalopathy (BSE) in cattle (in particular cows), and its form in humans variant Creutzfeldt–Jakob disease (vCJD), which was the consequence of food exposure to BSE and transmission of the infectious proteins typically called PrP^{TSE}. Human-to-human transfusion-associated transmission of vCJD has been recorded in four instances, in association with the transfusion of single-donor nonleukocyte-reduced red blood cell concentrates between 1996 and 1999 [39,40]. Besides a suspicious case of transmission of vCJD in a hemophilia A patient, possibly linked to the transfusion of a low-purity pooled factor VIII concentrate in the UK [41], there is no reported transmission of any TSE by industrial human plasma products, although continuous surveillance is in place [42]. The apparent safety of pooled fractionated plasma products may be due to several reasons. There is a very low level of infectivity (femtomolar range) of PRP^{TSE} in plasma. In addition, incidental partitioning and removal occur during production steps such as depth filtration, chromatography, and nanofiltration, which were found, through spiking experiments, to remove experimental models of PrP^{TSE} [43]. Any theoretical concerns regarding the risks of transmission of PrP^{TSE} by pooled HPL are based on the fact that current HPLs are not fractionated or nanofiltered and because there is no barrier species. Precautionary measures to mitigate a possible prion risk include those in place for the production of PC: (i) deferral of donors presenting with potential risk factors (e.g., history of travel to BSE-affected countries and susceptible to having eaten contaminated beef products); and (ii) implementation of universal leukocyte reduction to deplete B lymphocytes that may disseminate prions [42]. In addition, epidemiological surveillance of blood donors and of blood product recipients remains essential to

identify any risk of blood-borne TSE transmission. Also, there is no indication that blood products may transmit any classic form of CJD prion disease (sporadic, genetic, or iatrogenic) [44]. Therefore, as continuous surveillance of blood product safety is in place, no specific measures related to spontaneous CJD applicable to HPL seem to be required at the moment.

A Need for HPL Products Dedicated to Specific Applications in Cell Therapy?

While several studies indicated that HPLs perform better than FBS in expanding and maintaining human MSCs [1], further research is required to unveil a possible discrete impact of different HPL preparations on expanded MSCs, such as gene expression and differentiation potential. The need for specific HPL products dedicated to some applications in cell therapy or better suited for specific cell types, including differentiated cells, may exist. Besides, as the use of MSC-derived extracellular vesicles (EVs) as standalone preparations for clinical applications has generated great clinical and industrial interest [45], there may be a need to make EV-depleted HPL commercially available, so that the MSC EVs are not 'contaminated' by EVs originating from the HPL supplement. The availability of EV-depleted HPL would allow better delineation of the clinical benefits of MSC EVs not contaminated by platelet-derived EVs. Procedures to deplete platelet-derived EVs from HPL may include ultracentrifugation [46] or ultrafiltration [47], but the development of alternative industrial-scale procedures providing efficient EV removal should be encouraged.

Impact on the Availability of PCs for Transfusion?

Accumulating experimental evidence confirms that HPL from expired PC can be used for the expansion of various human cell types, with no evident decline in potency compared with HPL made from freshly collected platelets [1,9,37]. Considering that, for instance, in the USA 11–24% of PC have expired and been discarded in recent years [7], the current supply of expired PC should be sufficient to cover the needs of HPL for clinical-grade applications in cell therapy and regenerative medicine. Therefore, the developing need for HPL should not compete with or affect the availability of PC for transfusion. The Working Party recommends the use of expired PC as source material for HPL, additionally avoiding the wastage of platelet products by blood establishments [48,49].

Concluding Remarks and Road Map for the Future

There is strong evidence of the successful and safe use of HPL as a FBS substitute for the animal-serum-free expansion of human cells for clinical transplantation and applications in tissue engineering. Serum-converted HPL without the need for heparin addition can be used to ensure xenofree culture conditions. It is striking that most studies show robust superiority of HPL over FBS despite variations in lysis/activation procedures for platelets and modes of production including the types of pathogen and/or dedicated virus reduction treatment. Although the set of quality control criteria required to characterize the various types of products and guide their release on the market should reach a consensus, current findings support the fact that, once remaining issues are fully addressed (see Outstanding Questions), cell therapy and the biotechnology industry can soon benefit from a range of standardized and safe HPL products.

Therefore, as HPL is becoming the preferred growth medium supplement for therapeutic cell expansion, the Working Party feels it to be important that measures are established to ensure that HPL, as a biological material from human origin, meets all so-far-known criteria for safety and efficacy. Thus, HPL manufacturers should ensure that PC used for its production are not of an inferior quality to those used for transfusion. Based on a risk assessment, HPL manufacturers should also consider which additional virus testing at the HPL-pool level (e.g., for HAV, HEV, or B19V) may have to be implemented, having in mind the number of donations pooled and the efficacy of any pathogen/virus inactivation procedure in place. The design and engineering criteria for HPL manufacturing facilities should meet GMP principles similar to those currently effective for the plasma industry or as described recently for the manufacture of therapeutic-grade EVs [45]. Special attention should also focus on ensuring correct flows (e.g., product, operators, waste) as well as careful process and equipment segregation, operator training, and operating procedures; process segregation is especially relevant

Outstanding Questions

What is the best set of quality control assays as criteria for the release of HPL to the market?

What is the optimal combination of virus reduction treatment to implement for optimal virus safety of HPL?

Do extracellular vesicles play a role in the functional activity of HPL for human cell propagation?

Can different HPL preparations result in preferential expansion of different cell types or induce subtle physiological specificities in expanded cells?

to avoid cross-contamination prior to versus after pathogen inactivation or removal steps. Single-use equipment for processing small-scale batches can be of value to avoid the need for cleaning and sanitization validations [50]. Quality production of HPL should be guaranteed by a quality assurance system compliant with national and international GMP regulations for cell-based medicinal products and biologicals [51,52]. As for any pharmaceuticals, and even more for therapeutics of human origin, the concept of traceability and look-back should be in place. Competent authorities are thus expected to play a crucial regulatory role in overlooking the quality, safety, and use of HPL for the propagation of therapeutic cells or cell-derived EVs. Stakeholders involved in the production of HPL should follow the same stringent quality and safety requirements as those already in place for all human blood-derived products.

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Disclaimer Statement

The definitions given in the Glossary are applicable in the context of this opinion article and may have different meanings in other contexts.

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