Highlights

- Human platelet lysate (HPL), made from even expired platelet concentrates is an effective novel growth medium supplement for xeno-free *ex vivo* propagation of human cells for cell therapy and regenerative medicine.
- A consensus is needed to ensure consistent quality and safety of HPL supplements with regards to (a) the source of platelet concentrates, (b) the pool size to mitigate donors' platelets variability, (c) the validation and standardization of manufacturing processes and (d) the minimum release criteria before marketing.
- It is critical to guarantee HPL pathogen safety through the implementation of complementary safety measures encompassing the screening of blood donors, the pathogen testing of platelet concentrates, and, for large pools, the implementation of dedicated virus reduction treatments.
- International consensus between the various stakeholders (blood establishments, the biotechnology industry, and regulators) seems close to delineating the important quality and safety criteria needed to make HPL a reliable new gold standard supplement for cell-based medicinal products.
- The development of functional correlates for the various cell types supported by HPL is needed.

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1 Opinion

2 Production and Quality Requirements of Human Platelet Lysate

3 *- A position statement from the Working Party on Cellular Therapies of the International Society of*
4 *Blood Transfusion -*

5

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29 **Running title:** Human platelet lysate: ISBT WP Opinion

30 **Keywords:** human platelet lysate; cell propagation; cell therapy; mesenchymal stromal cells

31 **Word count** (core text): 3170

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34 **Website link: http://isbt-web.org/working-parties/cellular-therapies/topics-and-activities**

35 **Abstract (120 words)**

36 Human platelet lysate (HPL), rich in growth factors, is an efficient alternative supplement to
37 fetal bovine serum for *ex vivo* propagation of stromal cell-based medicinal products. Since
38 2014, HPL is a focus of the Working Party for Cellular Therapies of the International Society
39 of Blood Transfusion (ISBT). Currently, as several GMP compliant manufacturing protocols
40 exist, an international consensus defining the optimal modes of industrial production, product
41 specification, pathogen safety and release criteria of this ancillary material is needed. This
42 opinion paper by the ISBT Working Party summarizes the current knowledge on HPL
43 production and proposes recommendations on manufacturing and quality management in line
44 with current technological innovations and regulations of biological products and advanced
45 therapy medicinal products.

46

47

Glossary

Ancillary material: 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 an HPL product meeting intended quality specifications for clinically-related application of the cell product.

Fetal bovine serum: 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 practices (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 (MSC): Stromal cells with the potential to differentiate into at least three lineages of cell types. MSC can be cultured in HPL-supplemented medium.

Platelet: An anucleated blood cell of 2 to 3 μm and a life-span of 7 to 10 days in the circulation that is instrumental in the control of hemorrhage. Platelets contain numerous factors (growth factors, cytokines, coagulation factors, etc.) essentially contributing to tissue repair and regeneration.

Platelet concentrate: 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 dysfunctions 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 platelet concentrates.

Plateletpheresis: Automatic dedicated collection procedure (also called thrombocytapheresis) assisted by an apheresis machine whereby blood is taken from a donor, separated by physical means to recover a concentrate of platelets that is suspended in 100% plasma or a mixture of plasma and platelet additive solution.

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 "transmissible spongiform encephalopathies" (TSEs), such as mad cow disease, and variant Creutzfeld Jakob disease 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.

Pathogen inactivation: A dedicated and validated treatment specifically intended to destroy the infectivity of pathogens (such as viruses, bacteria, or parasites) that may be present in starting platelet concentrates or in HPL.

Pathogen removal: A dedicated and validated production treatment specifically intended to remove bacteria or viruses that may be present in HPL.

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.

Xeno-free: Terminology used to define that all components in the culture medium derived from the same species as the cells, i.e., for human cells this means free of animal-derived components.

48

49 **Definition and use of human platelet lysate (HPL)**

50

51 Human **platelet lysate** (HPL) is a cell-free, protein and growth factor-rich, biological material
52 that is produced mostly from expired clinical-grade human **platelet concentrates** (PC) initially
53 intended for transfusion. Numerous studies have now proven that HPL is, in particular, an
54 excellent clinical-grade supplement of growth media used for the phases of *in vitro* culture and
55 expansion of therapeutic cell-based medicinal products [1-3]. HPL is, therefore, emerging as an
56 efficient substitute to **fetal bovine serum** (FBS) as **xeno-free** growth medium supplement; its
57 human origin alleviates immunological and infectious safety concerns associated with the use of
58 materials from bovine origin. It also provides a currently suitable **good manufacturing**
59 **practices** (GMP)-compliant alternative to "chemically-defined" media when those are not
60 efficient for the culture of primary cells [1, 4, 5]. When compared to FBS for the expansion of
61 **mesenchymal stromal cells** (MSC) from various tissues, use of HPL generally results in better
62 cell expansion, shorter doubling time as well as maintenance of cellular immunophenotype,
63 immunosuppressive function, and differentiation capacity. Moreover, these expanded cells did
64 not stimulate tumorigenicity *in vivo* [1]. Considering the role HPL is going to play in the
65 biotechnology and cell therapy industry, the impetus to achieve consensus on quality and safety
66 criteria is urgently needed.

67 **Human platelet concentrates as source material for HPL production**

68 HPL is produced from PC initially intended for transfusion purposes. PC can be prepared from
69 200-500 mL anticoagulated **whole blood donations**, as a by-product of the preparation of red
70 blood cell concentrates and plasma, or using a dedicated automatic platelet collection procedure
71 called **plateletpheresis**. In the PC (app. 150-300 mL) platelets are enriched 4 to 5 fold compared
72 to the physiological level in the blood circulation. To reach the required amount of platelets
73 suitable for transfusion, and a sufficient hemostatic effect in patients, the "**buffy coat**" units of 4
74 to 6 whole blood-derived platelet donations are pooled to prepare a therapeutic dose for adult
75 patients. For single donor PC from plateletpheresis, no pooling is needed. The platelets can be
76 suspended in plasma, or in a mixture of plasma and platelet additive solution (PAS). According
77 to the Council of Europe 'Guide to the preparation, use and quality assurance of blood
78 components' [6] the minimum platelet content should be 2×10^{11} with less than 1×10^{6} residual
79 leukocytes per therapeutic unit. The pH should be more than 6.4 at the end of the shelf life. PC
80 must be tested negative for microbiological contaminations as bacteria and fungi. Table 1
81 summarizes the set of quality control of platelet concentrates as raw materials to produce HPL
82 recommended by the Working Party.

83 Only regular, traceable, clinical-grade PC produced by licensed **blood establishments** should be
84 used as source material for HPL. Acceptable variations are the following: (a) PC may be derived
85 from apheresis, platelet-rich plasma or pooled buffy coats; (b) PC may contain 100% plasma or
86 30-40% plasma and 60-70% PAS; and (c) PC may be gamma-irradiated (30Gy) or may be
87 pathogen-reduced by techniques licensed for this product (see below). As plasma of female
88 donors may contain antibodies directed against HLA (human leukocyte antigens) and HNA
89 (human neutrophil antigens) from previous pregnancies, using PC only from male donors or
90 negatively tested female donors may be considered to prevent any possible transmission to the
91 recipients of expanded cells. It is however still unknown whether such a risk does exist.

92 The shelf-life of PC, which is guided by the need to limit the risk of bacterial growth, is 5 to 7
93 days at 22 ± 2°C, depending upon legislation. This short shelf-life, associated with the need to
94 ensure a sufficient inventory of platelets for transfusion medicine, leads to the fact that 10-20%
95 of produced PC cannot be transfused, and are thus discarded [7]. PC not transfused within this
96 time frame of their collection can be frozen and used as raw material to prepared HPL without
97 noticeable impact on subsequent cell propagation [8, 9]. The committee believes that freezing of
98 PC within seven days after collection for further manufacture into HPL is readily feasible by
99 blood establishments and contributes to limiting the risk of bacterial growth. However, the
100 maximum period time PC can be used after expiry to prepare an efficient HPL for cell expansion
101 is unknown.

102 **Methods for HPL manufacture and pool size to ensure consistency**

103 Various protocols for platelet lysate generation and further processing of HPL are available from
104 the literature, and a comprehensive overview exposed in a recent review [1]. The **pooling** of a
105 sufficient number of PC is intended to counterbalance possibly fluctuant growth factor
106 concentrations in different PC donations. In a recently published international survey [2], all
107 participating centers stated to prefer pooling to avoid previously reported donor variations [10,
108 11]. However, even for GMP-grade manufacture, the number of pooled lysates was reported to
109 be highly variable (from 4 up to 125 platelet concentrate units) [2]. Other published studies have
110 used a pool size of 10 to 15 PC, corresponding to 40 to 50 individual donations [12, 13] to
111 provide a sufficient level of standardization. Besides, as described below, due to the risk of
112 transmission of pathogens, the number of pooled blood products should be limited, as
113 recommended by the European Pharmacopoeia (Chapter 5.2.12) [14] unless HPL undergoes
114 pathogen inactivation or reduction. Also in the U.S. Pharmacopeia (Chapter <1043>) [15] a
115 qualification program for **ancillary materials** (AM) as platelet lysate and a risk-based approach
116 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. 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 treatment of platelet concentrates (2%) or 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/+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 (a) the use of PAS during the preparation of PC, and (b) 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 a depletion of fibrinogen and coagulation factors. It also enables the avoidance of heparin that is otherwise needed for preventing 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 to promote MSC expansion [1]. Therefore, the Working Party does not recommend one production method of HPL over another. When concerns exist about the use of animal-derived heparin [18] to counter-balance the presence of coagulant fibrinogen, serum-converted HPL should be preferred.

Quality control parameters of HPL

Standardization of quality control and **release testing** of HPL has to consider regulatory requirements for raw materials or AM, as well as product specifications defined by the manufacturer. The United States Pharmacopeia (USP) 37 describes in the 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 platelet concentrates 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 Pharmacopeia (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 polymerase chain reaction (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 the 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 applying 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 specification of HPL suggested by the Working Party. The range and values indicated for proteins, including growth factors, may be product-specific, being dependent upon the PC source and the HPL production process.

HPL pathogen safety

Several types of donor blood-derived pathogens can contaminate freshly-collected PC: bacteria (such as spirochete, the agent of syphilis), parasites (such as Babesia microti; plasmodium; leishmania; or Trypanosoma cruzi), or viruses. This can be a major cause of transfusion-transmitted infections (TTI) as the storage of PC at 22 ± 2°C for up to five or seven 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 transmissions 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 to PC for transfusion, is mitigated by the fact that several PC freeze-thaw cycles implemented for platelet lysis contribute to 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 regards to HPL safety. Human blood products can transmit viral infectious agents, including lipid-enveloped viruses (human immunodeficiency virus, HIV; hepatitis B virus, HBV; hepatitis C virus, HCV; and various emerging viruses such as West Nile virus, WNV; dengue virus, DENV; Zika virus, ZIKV; etc.) and non-enveloped viruses (hepatitis A virus, HAV; parvovirus B19, and hepatitis E virus, HEV) [23-25]. Historical perspectives with clinical use of industrial plasma-derived coagulation factors indicated 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 (a) strict screening of blood/plasma donors, (b) virus testing of blood/plasma donations, and, most importantly, (c) introducing validated robust **pathogen inactivation** and **pathogen removal** steps during the production process [23, 26] (Fig. 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 to trace back any quality and safety problems, including infectious risks, and to take measures needed to protect both the blood donors and the recipients of the expanded cells.

216 Based on historical perspectives and current knowledge about the main blood-borne viruses, the
217 spectrum of measures recommended to diminish the infectious risks of pooled blood products are
218 listed in Table 4. Applying similar measures is vital for HPL as it is used for human cell
219 propagation, with broader categories of patients in the regenerative medicine field exposed to
220 blood-derived products compared to now. The European Pharmacopoeia recommends a
221 limitation in the number of donations pooled, when there are no virus inactivation or removal
222 steps, but does not actually give a specific recommendation of the pool size [14]. In contrast, for
223 instance, German regulations by the Paul Ehrlich Institute (PEI) specify the restriction to 16
224 individual donations when no virus inactivation treatment is applied [29]. One virus inactivation
225 approach is to use PC subjected to a "pathogen reduction" treatment. PCs "pathogen-reduced" by
226 psoralen/UVA [30-32] or by short-wave UV light [33] have been found suitable to prepare HPLs
227 for the propagation of various types of human MSCs. A second approach consists in
228 implementing a dedicated HPL virus inactivation treatment during **downstream processing**.
229 Solvent/detergent (S/D) treatment, which effectively inactivates lipid-enveloped viruses, can be
230 applied without affecting HPL capacity to expand adipose-tissue [34] and bone marrow [35]
231 derived MSC. Gamma irradiation of HPL is a recently proposed procedure that provides efficient
232 inactivation of a broad range of viruses without affecting the ability of HPL supplement for MSC
233 expansion [36]. As viral reduction treatments inherently have limitations in the extent or range of
234 virus inactivation, combining approaches, such as psoralen/UVA and S/D, may provide an
235 additional margin of safety needed for industrial-scale HPL pools, as shown recently [37].
236 Another means is to test the starting HPL manufacturing pool by NAT, as done in the plasma
237 fractionation industry, to verify either the absence of known virus markers or the presence of
238 neutralizing antibodies to infectious viruses.
239 In conclusion, the Working Party suggests that applying at least one step of virus inactivation
240 should be a mandatory trend for pooled HPL used for the propagation of therapeutic human cells
241 when the pool size is above 16 individual donations, in line with the recommendations of the PEI
242 [29]. However, the maximum pool size acceptable should be defined based on risk assessment
243 taking into account epidemiology, donor screening, and virus testing strategies. The
244 implementation of a dedicated virus reduction step can allow the processing of larger pool size.
245 As a possible reference, the maximum allowed pool size of industrial S/D-treated plasma for
246 transfusion (a therapeutic product that, like HPL, is not subjected to fractionation steps) varies
247 upon legislation. It was set at 60 liters in France, 200 liters in Germany and South Africa, 380
248 liters in Austria and up to 650 liters in the USA [38]. The need to combine two "orthogonal"
249 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, HPL pool size, and the extent of pathogen removal or inactivation achieved by measures in place.

HPL prion safety

Prions are very resistant unconventional infectious misfolded proteins mostly present in the central nervous system and responsible for transmissible spongiform encephalopathies (TSE). TSE include bovine spongiform encephalopathy (BSE) in cattle (in particular cows), and its form in human, 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]. Apart from 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]. 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 takes place 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 is based on the fact that current HPLs are not fractionated nor nanofiltered, and because there is no barrier species. Precautionary measures to mitigate a possible prion risk include those in place for the production of PCs: (a) deferral of donors presenting potential risk factors (such as history of travel to BSE-affected countries and susceptible to have eaten contaminated beef products) and (b) implementation of universal leukocyte reduction to deplete B lymphocytes that may disseminate prions [42]. In addition, epidemiological surveillance of blood donors and of blood products 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, and 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 to expand and maintain

283 human MSCs [1], further research is required to unveil a possible discrete impact of different
284 HPL preparations on expanded MSCs, such as gene expression and differentiation potential.
285 The need for specific HPL products, dedicated to some applications in cell therapy or better
286 suitable for specific cell types, including differentiated cells, may exist. Besides, as the use of
287 MSC-derived extracellular vesicles (EVs), as stand-alone preparations for clinical applications,
288 has generated great clinical and industrial interest [45], there may be a need to make EV-
289 depleted HPL commercially available, so that the MSC-EVs are not "contaminated" by EVs
290 originating from the HPL supplement. Availability of EV-depleted HPL would allow to better
291 delineating the clinical benefits of MSC-EVs not "contaminated" by platelet-derived EVs.
292 Procedures to deplete platelet-derived EVs from HPL may include ultracentrifugation [46] or
293 ultrafiltration [47], but the development of alternative industrial-scale procedures providing
294 efficient EV removal should be encouraged.
295
296 **<u>Impact on the availability of platelet concentrates for transfusion?</u>**

297 Accumulating experimental evidence confirms that HPL from expired PC can be used for the
298 expansion of various human cell types, with no evident decline in potency compared to HPL
299 made from freshly collected platelets [1, 9, 37]. Considering that, for instance in the USA, 11-
300 24% of PC have been expired and discarded in the recent years [7], the current supply of expired
301 PC should be sufficient to cover the needs of HPL for clinical-grade applications in cell therapy
302 and regenerative medicine. Therefore, the developing need for HPL should not compete nor
303 affect the availability of PC for transfusion. The WP recommends the use of expired PC as
304 source material for HPL, additionally avoiding the wastage of platelet products by blood
305 establishments [48, 49].

306

307 **Concluding remarks and road map for the future**

308 There is strong evidence of the successful and safe use of HPL as FBS substitute for the animal
309 serum-free expansion of human cells for clinical transplantation and applications in tissue
310 engineering. Serum-converted HPL without the need for heparin addition can be used to ensure
311 xeno-free culture conditions. It is striking that most studies show robust superiority of HPL to
312 FBS, in spite of variations in lysis/activation procedures of platelets, and modes of production
313 including the types of pathogen and/or dedicated virus reduction treatment. Although the set of
314 quality control criteria required to characterize the various types of products and guide their
315 release on the market should reach a consensus, current findings support the fact that, once
316 remaining issues are fully addressed (see "outstanding questions"), cell therapy and

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 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. As such, HPL manufacturers should ensure that PC used for its production are not of an inferior quality than those used for transfusion. Based on a risk assessment, HPL manufacturers should also consider, which additional virus testing at the HPL pool level (such as 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 of the HPL manufacturing facilities should meet similar GMP principles as 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, operators training, and operating procedures; process segregation is indeed especially relevant to avoid cross-contaminations prior to versus after pathogen inactivation or removal steps. Single-use equipment for processing small-scale batches can be of value to avoid the needs for cleaning and sanitization validations [50]. Quality production of HPL should be guaranteed by a Quality assurance (QA) system compliant with national and international GMP regulations for cell-based medicinal products and biological [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.

Disclaimer

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

Acknowledgments

Thanks are expressed to the International Society of Blood Transfusion for its support to the activities of the Working Party on Cellular Therapies.

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Figures legends

Figure 1: Overall production scheme of human platelet lysate. Platelet concentrates (PC) are prepared by licensed blood establishments from whole blood (WB) or are collected by apheresis. When reaching the expiry date the unused PC are frozen and transported to the HPL production facility. The PC are thawed, pooled, processed and dispensed into final human platelet lysate (HPL). The HPL is used as supplement of cell growth medium (GM).

Figure 2: Safety measures contributing to the ~~virus~~ pathogen safety of human platelet lysate. As for any blood products, the pathogen safety of human platelet lysate (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 donors screening) and the implementation of dedicated pathogen testing and pathogen (especially virus) inactivation and removal procedures.

498 **Table 1 Quality control requirements for platelet concentrates as starting materials for HPL production**

499

	Parameters	Specification range	Frequency of control
Infectious disease markers	Anti-HIV 1/2, HBV surface antigen, anti-HCV, HIV NAT, HBV NAT, HCV NAT, syphilis, WNV NAT* and ZIKV NAT*	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		> 40mL per 0.6 $x10^{11}$ platelets	As determined by SPC
Cell content	Platelets	> 2x 10^{11} / unit	As determined by SPC
	Residual leukocytes	$< 1 \times 10^{6}$ per unit	As determined by SPC

500 Modified from [6], * mandatory in the US

501 Abbreviations: HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; NAT, nucleic acid amplification
502 test; WNV, West Nile virus; ZIKV, Zika virus; SPC, statistical process control.

503

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505 **Table 2: Example of the protein content of HPLs, depending upon characteristics of PC and mode of platelet lysis (adapted from [1, 3]**

506

	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/CaCl2 treatment	**B1** Freeze-thaw	**B2** Thrombin/CaCl2 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		
	Immunoglobulin G, g/dL	0.8-1.2	0.8-1.2		
	Immunoglobulin A, g/dL	0.07- 0.13	0.07-0.13		
	Immunoglobulin M, 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			

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510 **Table 3 Example of a set of quality specifications of HPL**

	Parameters	Specification range	Method of testing	Frequency of testing
Pooling	Number of donations	10 - 16	-	-
Sterility	Bacteria and fungi	Negative	Automated microbial detection system, PCR or ELISA	All batches
	Endotoxin	< 0.5 EU/mL	LAL endotoxin tests [53]	
	Mycoplasma	Negative	Culture method [54]	
Biochemical analysis	Osmolality pH Total protein	According to the range of standard values of human blood	Osmometer pH-meter Biuret protein assay	All batches (specification for 100% plasma HPL) [14]
Immunology	Isoagglutinins	To be validated	ICT	All batches used for culture of ECFC
Potency and functionality	Platelet-derived growth factors according to [1]			Randomly
	PDGF-AB	> 50 ng/mL	ELISA	
	TGF-β1	> 50 ng/mL	ELISA	
	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

511 Abbreviations: HPL, human platelet lysate; PCR, polymerase chain reaction; LAL, limulus amebocyte lysate; EU, endotoxin unit (1 international unit
512 of bacteria endotoxin is according to1 [53]); ICT, indirect coombs test; ECFC, endothelial colony-forming cells; ELISA, enzyme-linked
513 immunosorbent assay.

514

Table 4: Current building blocks and additional virus safety steps considered for pooled HPL

(based on experience and regulations of industrial plasma products)

	Epidemiological surveillance	**Repeat donors**	**Donor's screening**	**Donation testing**	**Pathogen reduction treatment of PC****	**Manufacturing/mini-pool testing**	**Dedicated virus reduction step**	**Final product testing**
Status	Already in place for preparing platelet concentrates 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 viruses (e.g. ZKV, WNV, DENV, HEV)			HIV, HBV, HCV	All viruses***	HIV, HBV, HCV (HAV, B19)***	All viruses****	HIV, HBV, HCV markers
Objectives	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 of infections	To use only donations non reactive for anti-HIV, anti-HCV, HBsAg, and nucleic acid testing (HIV; HBV; HCV)*	To inactivate pathogens in PC	To ensure that the HPL manufacturing pool is non reactive for markers of known tested viruses	To inactivate a broad range of viruses in HPL. Two methods described: solvent-detergent and gamma-irradiation	To reconfirm that PC and HPL batch were non reactive for virus markers

* NAT testing may be mandatory for other viruses (e.g. ZIKV, WNV) in some legislation; ** Some pathogen reduction treatments are licensed in most HDI countries; ***potentially other viruses as needed; **** Technologies in use may have limits in their capacity to inactivate some viruses

Outstanding Questions

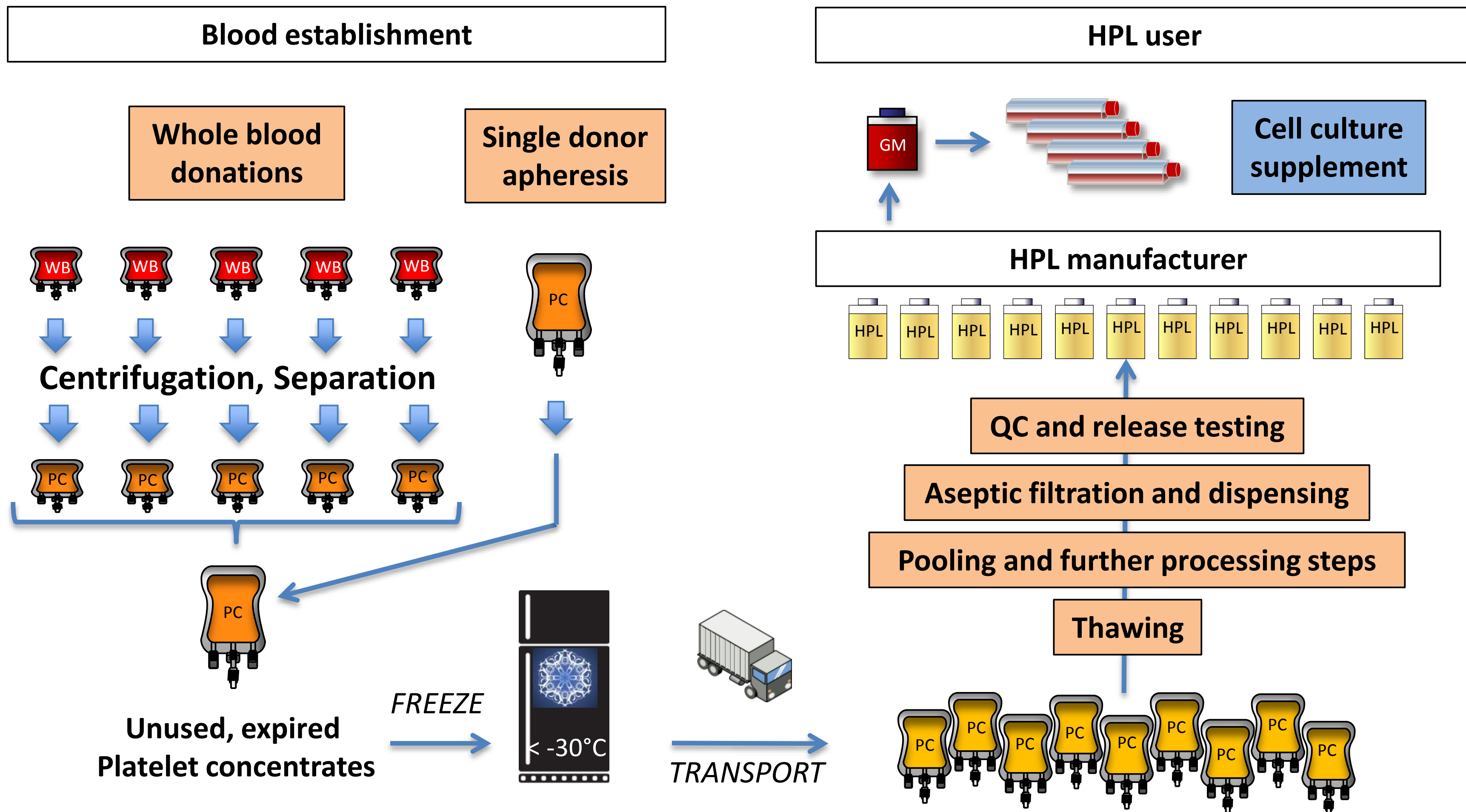
What is the best set of quality control assays as release criteria of HPL to the market?

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

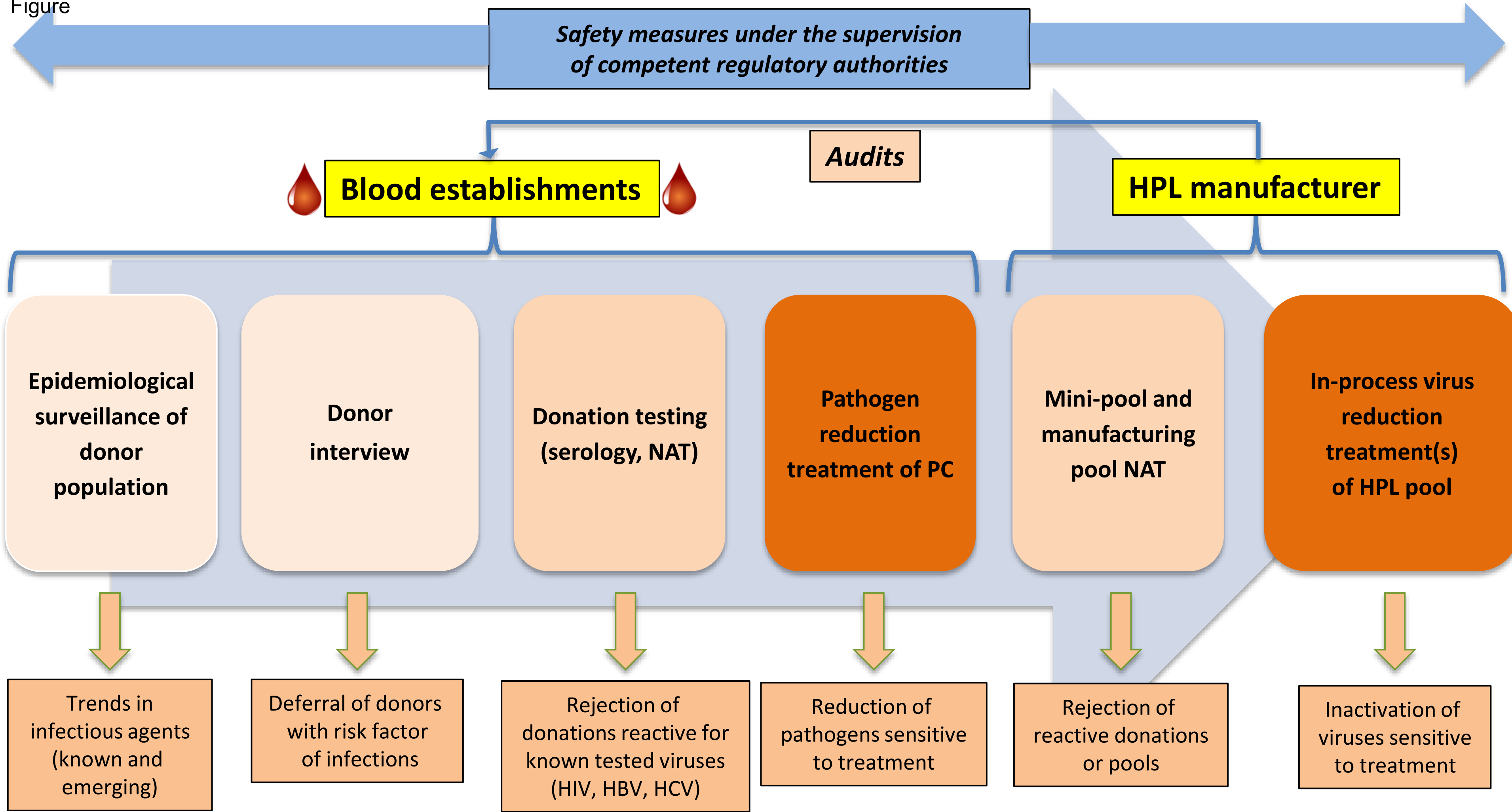
Do extracellular vesicles (EVs) play a role in the functional activity of HPL for human cell propagation?

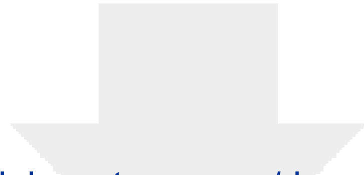
Can different HPL preparations result in a preferential expansion of different cell types or induce subtle physiological specificities to expanded cells?

Figure 1



Figure





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