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Standard Guide for Autologous Platelet-Rich Plasma for Use in Tissue Engineering and Cell Therapy[1]

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1. Scope

1.1 This guide defines terminology and identifies key fundamental properties of autologous platelet-rich plasma (PRP) and PRP-derived platelet gels intended to be used for tissue engineered medical products (TEMPS) or for cell therapy applications. This guide provides a common nomenclature and basis for describing notable properties and processing parameters for PRP and platelet gels that may have utility for manufacturers, researchers, and clinicians. Further discussion is also provided on certain aspects of PRP processing techniques, characterization, and quality assurance and how those considerations may impact key properties. The PRP characteristics outlined in this guide were selected based n a review of contemporary scientific and clinical literature but do not necessarily represent a comprehensive inventory; other significant unidentified properties may exist or be revealed by future scientific evaluation. This guide provides general recommendations for how to identify and cite relevant characteristics of PRP, based on broad utility; however, users of this standard should consult referenced documents for further information on the relative import or significance of any particular PRP characteristic in a particular context.

1.2 The scope of this guide is confined to aspects of PRP and platelet gels derived and processed from autologous human peripheral blood. Platelet-rich plasma, as defined within the scope of this standard, may include leukocytes.

1.3 The scope of this document is limited to guidance for PRP and platelet gels that are intended to be used for TEMPS or for cell therapy applications. Processing of PRP, other platelet concentrates or other blood components for direct intravenous transfusion is outside the scope of this guide. Apheresis platelets and other platelet concentrates utilized in transfusion medicine are outside the scope of this document. Production of PRP or platelet gels for diagnostic or research applications unrelated to PRP intended for TEMPS or cell therapy is also outside the scope of this guide. Fibrin gels devoid of platelets are also excluded from discussion within this document.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices (Withdrawn 2012)[3]

F2149 Test Method for Automated Analyses of Cells—the Electrical Sensing Zone Method of Enumerating and Sizing Single Cell Suspensions

F2312 Terminology Relating to Tissue Engineered Medical Products

2.2 *ISO Standards:*[4]

ISO 5725–1 Accuracy (trueness and precision) of Measurement Methods and Results—Part 1: General Principles and Definitions—Technical Corrigendum 1

ISO 5725–2:1994 Accuracy (trueness and precision) of Measurement Methods and Results—Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method—Technical Corrigendum 1

3. Terminology

3.1 *Definitions:*

3.1.1 *atuologous, adj*—cells, tissues, and organs in which the donor and recipient is the same individual. Synonyms: autogenous, autograft, or autotransfusion, a self-to-self graft. **F2312**

[1] This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.43 on Cells and Tissue Engineered Constructs for TEMPs.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

3.1.2 *biomolecule, n*—a biologically active peptide, protein, carbohydrate, vitamin, lipid, or nucleic acid produced by and purified from naturally occurring or recombinant organisms, tissues or cell lines or synthetic analogs of such molecules. A biomolecule may be used as a component of a TEMP. **F2312**

3.1.3 *cell therapy, n*—the administration of cells (any kind and form) to repair, modify or regenerate the recipient's cells, tissues, and organs or their structure and function, or both. Cell therapy technologies can be applied in tissue engineering to generate TEMPs. **F2312**

3.1.4 *device, n*—an instrument, apparatus, implement, machine, contrivance, implant, *in vitro* reagent, or other similar or related article intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended purposes. Devices are intended to affect the structure or any function of the body. **F2312**

3.1.4.1 *Discussion—Device Criteria*: A liquid, powder, or other similar formulation intended only to serve as a component, part or accessory to a device with a primary mode of action that is physical in nature. A device may be used as a component of a TEMP.

3.1.5 *donor, n*—a living or deceased organism who is the source of cells or tissues, or both, for research or further processing for transplantation in accordance with established medical criteria and procedures. **F2312**

3.1.6 *gel, n*—the three-dimensional network structure arising from intermolecular polymer chain interactions. **F2312**

3.1.6.1 *Discussion*—Such chain interactions may be covalent, ionic, hydrogen bond, or hydrophobic in nature. See also Terminology F1251.

3.1.7 *heal, v*—to restore wounded parts or to make healthy. **F2312**

3.1.8 *healing, n*—the restoration of integrity to injured tissue. **F2312**

3.1.9 *processing, vt*—any activity performed on cells, tissues, and organs other than recovery, such as preparation and preservation for storage and packaging. **F2312**

3.1.10 *recipient, n*—the individual or organism into whom materials are grafted or implanted. **F2312**

3.1.11 *recovery, n*—the obtaining of cells or tissues which may be used for the production of TEMPs. **F2312**

3.1.12 *regenerative medicine, n*—a branch of medical science that applies the principles of regenerative biology to specifically restore or recreate the structure and function of human cells, tissues, and organs that do not adequately regenerate. **F2312**

3.1.13 *suspension, n*—the dispersion of a solid through a liquid with a particle size large enough to be detected by purely optical means. **F2312**

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *activation, v*—conversion of a liquid platelet-rich plasma to a solid platelet-rich gel.

3.2.1.1 *Discussion*—In the context of platelet-rich plasma, activation can be passive or active. Passive activation is a typical consequence of removing blood from the circulatory system, the dynamics of which can influenced by platelet-rich plasma processing. Active activation is directed action intended to stimulate coagulation, for example, addition of an exogenous agonist or proactive reversal of anticoagulation.

3.2.2 *blood cell, n*—one of the formed elements of the blood; a leukocyte, erythrocyte or platelet. Also called blood corpuscle, hemacyte, hematocyte and hemocyte (**1**).[5]

3.2.3 *cell, n*—the smallest structural unit of an organism that is capable of independent functioning, consisting of one or more nuclei, cytoplasm, and various organelles, all surrounded by a semipermeable cell membrane (**1**).

3.2.3.1 *Discussion*—For the purposes of this guide, the term cell includes all formed elements of the blood within the scope of the term "blood cell." Erythrocytes and platelets are anucleate in their mature forms, and therefore may not meet the strict definition for cell above. However, erythrocytes and platelets are considered or are frequently referred to as cells in platelet-rich plasma applications so they are included in the broader scope of the term used for this guide.

3.2.4 *coagulation, n*—the sequential process by which the multiple coagulation factors of the blood interact in the coagulation cascade, ultimately resulting in the formation of an insoluble fibrin clot (**1**) .

3.2.5 *erythrocyte, n*—a mature red blood cell. Synonymous with red blood cell, red corpuscle (**2**).

3.2.6 *leukocyte, n*—a colorless blood cell capable of ameboid movement; there are several different types, classified into the two large groups granular leukocytess (basophils, eosinophils, and neutrophils) and nongranular leukocytess (lymphocytes and monocytes). Also called white blood cells or corpuscles (**1**).

3.2.7 *peripheral blood, n*—the blood in the systemic circulation (**1**).

3.2.8 *plasma, n*—the fluid portion of the blood in which the particulate components are suspended. Plasma is to be distinguished from serum, which is the cell-free portion of the blood from which the fibrinogen has been separated in the process of clotting. (**1**)

3.2.9 *platelet, n*—a disk-shaped structure, two to four micrometers (μm) in diameter, found in the blood of all mammals and chiefly known for its role in blood coagulation; platelets, which are formed in the megakaryocyte and released from its cytoplasm in clusters, lack a nucleus and DNA but contain active enzymes and mitochondria. Also called thrombocyte (**1**).

3.2.10 *platelet concentrate, n*—a blood-derived suspension or gel in which the majority of erythrocytes have been removed

[5] The boldface numbers in parentheses refer to the list of references at the end of this standard.

and platelets have been concentrated with respect to normal physiological levels or with respect to the source blood prior to processing.

3.2.10.1 *Discussion*—This definition relates to the term platelet concentrate as generally applied within the context of cell therapy or TEMPs applications. This definition does not necessarily extend to applications within hematology, transfusion medicine, or other fields.

3.2.11 *platelet-rich plasma, n*—a blood-derived plasma suspension from which the majority of erythrocytes have been removed and platelets have been concentrated with respect to normal physiological levels or with respect to the source blood prior to processing. Commonly abbreviated PRP.

3.2.11.1 *Discussion*—This definition relates to the term platelet-rich plasma as generally applied within the context of cell therapy or TEMPs applications. This definition does not necessarily extend to applications within hematology, transfusion medicine, or other fields.

3.2.12 *platelet gel, n*—a platelet-rich plasma-derived gel. Platelet gels are formed from platelet-rich plasma through passive or directed activation of coagulation.

3.2.12.1 *Discussion*—This definition relates to the term platelet gel as generally applied within the context of cell therapy or TEMPs applications. this definition does not necessarily extend to applications within hematology, transfusion medicine, or other fields.

3.2.13 *serum, specifically blood serum, n*—the clear liquid that separates from the blood when it is allowed to clot completely. It is therefore blood plasma from which fibrinogen has been removed in the process of clotting (**1**).

4. Significance and Use

4.1 Autologous PRP and platelet gels are utilized in a wide range of orthopedic, sports medicine, regenerative medicine, and surgical applications (**3-5**). PRP and platelet gels are layered, sprayed, injected, molded, or packed, alone or in combination with graft material or TEMPs, into a variety of anatomical sites, tissues, and voids (**3, 6**). These platelet concentrates can provide an assortment of bioactive molecules, cells, and physical properties that are potentially attractive for promoting healing and other cell therapy applications (**7**). Unfortunately, the term "platelet-rich plasma" or "PRP," which is ubiquitous in early and contemporary medical literature related to a variety of platelet concentrates, only unambiguously denotes one critical parameter of a platelet suspension—increased platelet concentration. Without further context, this common description of PRP offers no information about other important physical and cellular aspects of platelet concentrations. As scientific and clinical understanding of PRP and other cellular therapies increases standardization of nomenclature and terminology is critical for defining key properties, standardizing processing parameters and techniques, and developing repeatable assays for quality assurance and scientific evaluation (**5, 8-13**). This guide outlines basic guidelines to describe key properties of unique PRP and platelet gel formulations in a standardized fashion. Reliable, standardized descriptions can provide valuable context to PRP end users, such as clinicians seeking a PRP or platelet gel with certain biological attributes or scientific investigators seeking to duplicate a published formulation or to correlate a given PRP or platelet gel feature to other biological properties or outcomes.

5. Key Properties of PRP and Platelet Gels

5.1 The physical and biological properties detailed in this section have been identified in peer-reviewed scientific articles or medical texts as factors that may potentially impact the safety and/or effectiveness of PRP and platelet gels used for TEMPS, cell therapies, or related applications. While the significance of individual properties relative to other properties is beyond the scope of this guide, recommendations are included for attributes which are consistently identified as significant throughout the literature. Unless otherwise noted, a parameter value or range quoted in this text is intended to represent the average value/range for a particular PRP or gel output; values should be expressed as mean ± standard deviation. A table of key properties appears in Annex A1, Table A1.1.

5.1.1 *Processing Volume:*

5.1.2 The whole blood input volume or input volume range for a given processing methodology or technology should be reported in milliliters (mL). Input volume or volume range should be specified to ensure proper processing technique. Furthermore, factors such as patient size or patient pathology may impact the amount of peripheral blood available for PRP processing, therefore minimal volume requirements can be useful information for end users. Processing volume should be qualified where appropriate, for example: mL per device or mL per processing tube. Processing volume requirements for a given PRP method should represent the value or range necessary to consistently produce PRP possessing the unique key properties reported for that PRP method, as detailed throughout this guide, recognizing any limitations inherent to the technology or specific to recommended processing accessories.

5.1.3 *Deliverable Volume:*

5.1.4 The mean volume of deliverable PRP output from a given processing methodology should be reported in milliliters. Providing deliverable output volume allows the end user to project if a given processing technique/device will provide sufficient deliverable material for their application and/or how many devices will be needed for a given procedure or evaluation. Platelet gel volume can be estimated from PRP volume, if necessary. Furthermore, the deliverable volume is significant because it can be multiplied with concentration descriptors to estimate the total amount of a given parameter to be delivered to a target. For instance, deliverable volume can be multiplied by the mean volumetric cell concentration for a given cell type to obtain the total number of those cells that can be delivered. Total cell number may be as important as cell concentration for some applications (**12**). The same principle hods for plasma constituents where the total amount delivered is of interest (**14**).

5.2 *Cellular Content:*

5.2.1 The impact of cellular content on PRP and platelet gel utility and activity is actively debated, and therefore of particular interest to PRP and platelet gel users and researchers.

The types of cells that make up a given PRP formulation, along with their relative concentrations within the suspension, are routinely identified as fundamentally critical characteristics of PRP and platelet gels. The scope of this guide is limited to platelet suspensions derived from peripheral blood, therefore three cell populations of primary interest: platelets, leukocytes and erythrocytes.

5.2.2 Accurate and repeatable cell identification and counting methodologies are necessary for meaningful descriptions of cellular content. These considerations are briefly discussed in 7.5.

5.2.3 *Platelet Concentration and Quantity:*

5.2.3.1 Platelets are notable because of their primary role in hemostasis and coagulation, their participation in wound healing activities, and their releasable internal stores of cytokines and growth factors. Any cellular suspension labeled PRP should, by definition, have an increased concentration of platelets relative to baseline. However, the advantages or disadvantages of particular PRP platelet concentrations in particular clinical applications is still an active area of research (**12**). Platelet concentration is therefore recognized by consensus as an important PRP descriptor. The mean volumetric platelet concentration within the final PRP suspension should be included in any formulation description. The concentration should be provided in platelets/microliter.

5.2.3.2 The fold increase in platelet concentration relative to the baseline platelet concentration of unprocessed blood from the same harvest should also be reported. Fold increase is recommended as an additional descriptor to platelet concentration, rather than a substitute, as baseline platelet concentrations can vary widely between individual patients/donors and even within the same patient/donor over time. However, fold concentration increase is useful for estimating the general efficiency of a specific methodology with respect to selecting or concentrating platelets. Blood is harvested conservatively as a general rule, but certain patient/donor populations may require special consideration where the efficiency of the processing technology adds value by minimizing peripheral blood depletion. Fold concentration increase can also be useful when comparing across preparation methods.

5.2.4 *Leukocyte Concentration and Quantity:*

5.2.4.1 The presence or absence of leukocytes in a PRP suspension, as well as the relative abundance of various types of leukocytes should be considered when characterizing and describing any PRP or platelet gels utilized for cell therapy or TEMPS. The role and significance of leukocytes in PRP is an ongoing topic of research and discussion, complicated by the fact that different types of leukocytes have widely variable functions in inflammation, healing, and immune response which can be tissue- and/or application-specific (**5**, **12**). Nevertheless, the presence of leukocytes and/or leukocyte concentration is widely regarded as a key parameter for PRP classification systems.

5.2.4.2 PRP descriptions should, at minimum, indicate the volumetric concentration of total leukocytes in the final output using units of cells/microliter. Following the same rationale outlined for platelets, fold concentration increase/decrease relative to baseline should also be noted. Differential leukocyte concentrations and fold increases can also be provided, when available. Differential concentration data can be divided into granulocytes, lymphocytes and monocytes or further detailed to differentiate between basophils, eosinophils and neutrophils within the granulocyte subpopulation.

5.2.5 *Erythorocyte Concentration:*

5.2.5.1 The presence of erythrocytes in PRP or platelet gels has been cited as detrimental for some cell therapy applications (**12**). PRP processing technologies typically aim to reduce the concentration of erythrocytes in the final PRP output or remove them completely. PRP erythrocyte concentration should be reported in erythrocytes/microliter. Fold concentration increase/decrease can also be provided as a supplemental descriptor. If significant hemolysis is observed, serum/plasma free hemoglobin should be reported in milligrams/deciliter.

5.3 *Activation State:*

5.3.1 The activation state of PRP can drastically influence the physical and biological activity of the output. Activation refers to conversion of soluble fibrinogen to polymerized fibrin by means of the coagulation cascade. Activation can be initiated by introduction of an external stimulus (for example, calcium chloride, bovine thrombin, concentrated autologous thrombin, etc.) or allowed to proceed naturally.

5.3.2 The exact manner of activation should be detailed to maximize repeatability and so that activation-dependent biological properties can be considered. Timeframe of activation should also be specified. Once activation is initiated, polymerization, cellular interactions, and cellular secretion events vary over time and impact the biological activity. The timeframe between activation initiation and end use should be provided when describing a processing technique or technology. End use would include application to a target site for clinical use or assay initiation for characterization studies.

6. Other Notable Properties

6.1 The properties detailed in this section are of special interest in certain applications and circumstances. They are recommended as supplemental descriptors for PRP where deemed useful.

6.2 *Fibrinogen Concentration:*

6.2.1 If plasma fibrinogen is significantly concentrated or depleted within a PRP, the extent of change may impact the physical properties of fibrin gel formed upon proactive or natural activation of the solution (see 5.5) (**8**). Fibrinogen concentration can be measured by the Clauss assay, prothrombin time-derived assay, or immunological assay (**15**). If reported, fibrinogen concentration should be reported in mg/dL or µg/mL.

6.3 *Growth Factors, Cytokines, and other Biomolecules:*

6.3.1 Growth factors, cytokines, and other biomolecules may be present at concentrated levels in certain PRP and platelet gel formulations (**7**). Quantitative and qualitative study of these molecules in PRP is still a developing field; however, quantitative data may be useful in some instances. Growth factor content and release can be dependent on the cellular makeup of the PRP, method and time course of PRP activation

(if any), and the resulting fibrin architecture (**8**). Immunological assays are recommended for quantitative measurement of common growth factors, cytokines, or biololecules. If levels are reported, units of concentration should be clearly outlined and the assay method should be briefly described or referenced, if possible. In particular, clear notation on whether the concentration is an extracellular value from the plasma or serum, or if it includes total levels from lysed cellular content, is valuable. The location of biomolecules within or outside of the cell may impact activity and or time course of effects *in vivo*. The manner of lysis should be noted, if applicable. If activation is utilized, the manner and timeframe should be reported.

6.4 *Percent Cell Recovery:*

6.4.1 The percent recovery of a given cell type can be a useful parameter for evaluating or comparing the efficiency of PRP processing methods. Percent recovery is the percentage of the total number a cell type in the PRP output relative to the baseline quantity of that cell in the initial input. For example, the PRP output from a PRP processing technique with 90 % platelet recovery would contain nine out of every ten platelets that were in the baseline input. Percent recovery can be used as a supplemental descriptor for any cell type that is deemed significant for a given methodology or application.

7. PRP Processing – Variables That May Affect Key Properties

7.1 This section identifies variables that may affect the core and supplemental description parameters outlined above. Understanding variables that impact how key properties are achieved, maintained, and/or modified is beneficial for thorough understanding and quality control of a given PRP or platelet gel. These considerations can be factored into PRP descriptions, product development, design of PRP-centered investigations, and technology selection. These considerations may also provide potential explanations for unforeseen variations in literature reports and experimental data. A table of significant considerations appears in Annex A1, Table A1.2.

7.2 *Donor/Patient Considerations:*

7.2.1 The scope of this standard is limited to autologous PRP and platelet gels. In clinical applications, the blood donor and PRP/platelet gel recipient are synonymous. Therefore, the biological state of the donor's peripheral blood at the time of harvest will impact the biological activity of the resulting PRP or platelet gel. For research studies, donor selection may be modified based on potential impact on experimental results, or study donor characteristics can be described so that potential impact can be considered by reviewers and users.

7.2.2 Initial cell counts in unprocessed harvested blood can affect the final PRP cell counts, depending on the system and technology. Abnormal hematological counts prior to a PRP procedure or a history of cell count anomalies should be considered prior to harvest and/or selection of PRP processing methodology.

7.2.3 Peripheral platelet counts can be clinically elevated (thrombocytosis) or, more commonly, decreased (thrombocytopenia) by a number of factors (**16**). Thrombocytopenia can be disease-induced, drug-induced, a function of decreased production, a function of increased sequestration, or a function of destruction/hemodilution during extracorporeal perfusion. Thrombocytosis can be the result of a primary thrombocytosis or a reaction to infection, chronic inflammation, malignancy or other factors.

7.2.4 Leukocyte and erythrocyte counts can also be outside normal ranges and affect PRP characteristics. The impact of anemias, sickle cell disease, leukocytopenias, leukocytosis, leukemias, lymphomas, infection, chronic inflammation, immunodeficiency, radiation exposure, chemotherapy and other natural and external modifiers of blood cell function and number should be considered.

7.2.5 PRP biological activity and cell function can also be affected by disease states and/or medications, particularly disorders or medications that influence coagulation, platelet function, platelet secretion, and leukocyte function. Common medications that impact platelet secretion include anti-platelet drugs (clopidogrel, ticagrelor, vorapaxar, etc.), aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) and anti-histamines (**17, 18**). When clinically appropriate, donors may refrain from medication for a period of time prior to the blood harvest, based on the pharmacodynamics and pharmacokinetic profile of the therapeutic, to reduce drug-induced effects on PRP. If drugs that inhibit platelet function are to be avoided, three days of abstinence prior to sampling are recommended for drugs known to reversibly inhibit platelet function, while ten days of abstinence are recommended for drugs that irreversibly inhibit platelet function (**19**).

7.3 *Blood Harvest Considerations:*

7.3.1 Blood draw should be through non-traumatic venipuncture utilizing best practices in phlebotomy (**20**). Only polypropylene or siliconized glass syringes should be used (**19, 21, 22**). A larger gauge needle should be used during blood draw, if possible, to prevent shear-induced platelet activation during the procedure. A 21 gauge or larger bore needle is recommended, however a smaller gauge may be more appropriate for pediatric or other special cases (**19, 20**).

7.3.2 Blood must be drawn into anticoagulant to prevent coagulation during PRP processing. Anticoagulant type and strength should be specified for a given PRP processing methodology, along with the appropriate volume to mix with a given volume of blood. Anticoagulant should be preloaded into the syringes used to collect the blood to minimize the delay until anticoagulation is initiated. If large syringes are used, they can be gently tilted during the draw so that mixing occurs. Gentle mixing of the entire sample, generally achieved by slow tilting of syringes or receptacles, should be performed as soon as possible after the draw to prevent time- and force-dependent activation.

7.3.3 Anticoagulant type and method is also important to document and consider because it can affect PRP activation dynamics and properties of the finished PRP or platelet gel (**22, 23**). Activation often relies upon or is affected by reversing the effect of the processing anticoagulant. For instance, citrate-based anticoagulants can be reversed by providing excess exogenous calcium to overwhelm the chelating effect of citrate. Calcium is utilized for the coagulation cascade and platelet activation pathways. Anticoagulants can also impact PRP pH and cell functions, whether directly or indirectly through

inhibition of another molecule, such as thrombin. Thrombin is not only the key enzyme in the final common pathway of coagulation, but is also a potent platelet agonist (**24**). Therefore, inhibiting thrombin generation with heparin can affect the dynamics of both coagulation and platelet activation. Heparin anticoagulation has also been reported to potentiate platelet activation and aggregation in other situations (**22, 25**).

7.4 *Processing Considerations:*

7.4.1 PRP and platelet gels are produced using a wide variety of processing technologies and methodologies. General considerations are provided here that can preserve biological activity and PRP/platelet gel quality.

7.4.2 The temperature of PRP or derived platelet gels should never be reduced below room temperature. Cold can promote platelet activation, agglutination, or spontaneous aggregation (**22**). These events can influence platelet secretion dynamics and affect platelet counting techniques, which may or may not be able to differentiate between single platelets and small aggregates.

7.4.3 The total time elapsed between blood harvest and clinical use or assay initiation should not exceed four hours. Platelets gradually become refractory once removed from the body and lose significant function after four hours (**19, 26**). This four hour time frame mirrors the time frame recommended by the AABB for platelet transfusion procedures (**27**). Fixation for later analysis can be appropriate for some assays and should be employed during the four hour window to capture relevant morphology or function (**28, 29**).

7.4.4 When centrifugation is utilized for cell concentration and/or separation, the centrifugation parameters in units of fold-increase over gravity (g) and length of time in minutes should be specified. If multiple stages of centrifugation are utilized, parameters should be specified for each stage. If column, membrane, gel, or other technologies are employed for concentration or separation, adequate specifications should be provided to adequately ensure the quality and reproducibility of the output.

7.5 *Cell Counting:*

7.5.1 Cell counting accuracy and reliability are crucial for maintaining the fidelity of the key descriptors related to cell concentration. Cell counts should be performed on a validated hematological analyzer or by an alternative validated method (**30-32**). Test Method F2149 discusses aspects of cell counting using the Coulter method. The International Organization for Standards (ISO) provides general guidance for the accuracy of measurement methods and results (**33, 34**). Two standards for cell counting are currently under development at ISO: ISO/NP 20391-1: Biotechnology - Cell Counting – Part 1, General Guidance on Cell Counting Methods and ISO/NP 20391-2: Biotechnology - Cell Counting – Part 2, Experimental Design and Statistical Analysis to Quantify Counting Method Performance. Cells should be gently suspended just prior to counting to avoid errors due to cell settling or plasma separation. The baseline used for fold-increase/fold-decrease calculations should be clearly defined. The recommended baseline is the cell concentration within the harvested blood following addition and mixing of anticoagulant. Baselines using normal physiological values or historical measurements on the same patient are discouraged, as cell counts can vary from day to day.

8. Keywords

8.1 cell and tissue engineering; cell therapy; platelet concentrate; platelet gel; platelet-rich plasma; processing of platelet-rich plasma; PRP; tissue engineered medical products

ANNEX

(Mandatory Information)

A1. KEY PROPERTIES AND SIGNIFICANT CONSIDERATIONS

A1.1 See Table A1.1.

TABLE A1.1 Key Properties for Describing PRP

	PRP Property	Units	Notes
Recommended Property Descriptors	Input Volume	___mL	Whole blood input volume per method/device
	Deliverable Output Volume	___mL	Deliverable output volume
	Platelet Concentration	___platelets/mL	
	Platelet Fold-Increase	___X	Relative to baseline platelet concentration
	Leukocyte Concentration	___cells/mL	Total leukocytes
	Leukocyte Fold-Increase	___X	Relative to baseline total leukocyte concentration
	Erythrocyte Concentration	___erythrocytes/mL	
	Activation State	PRP or Platelet Gel	Activated PRP is a gel at time of use or evaluation
	Additives or Reagents		List any extrinsic additives or reagents that are required for processing or are part of the final formulation, for example, anticoagulant or coagulation agent
Supplemental Property Descriptors	Fibrinogen Concentration	___mg/dL or ___mg/mL	
	Biomolecule Content	as appropriate	Growth factors, cytokines, proteins, etc.
	Percent Cell Recovery	___%	
	Differential Leukocyte Concentrations	___cells/mL	Leukocyte data broken down by leukocyte subtype

A1.2 See Table A1.2.

TABLE A1.2 Donor, Processing, and Reporting Considerations for PRP

Consideration	Recommendation
Donor/Patient Considerations	
Abnormalities in Cell Number or function	
Pathology/Disease	
Medication	
Blood Harvest Considerations	
Non-traumatic phlebotomy	
Syringe material	Polypropylene
Needle gauge	≥21 gauge
Anticoagulant	Application-specific
Processing Considerations	
Storage and processing temperature	Room temperature, no refrigeration
Time between harvest and use	≤4 h
Centrifugation parameters	Provide applied centrifugal force in fold increase over gravity (g), provide centrifugation time in minutes, provide sufficient description of multiple stages, if applicable
Reporting/Specification Considerations	
Accurate Cell Counting	Use validated method, standard baseline

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