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# Standard Guide for Autologous Platelet-Rich Plasma for Use in Tissue Engineering and Cell Therapy<sup>1</sup>

This standard is issued under the fixed designation F3209; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 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 on 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

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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:<sup>2</sup>

F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices (Withdrawn 2012)<sup>3</sup>

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:<sup>4</sup>

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 *autologous*, *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**

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

<sup>3</sup> The last approved version of this historical standard is referenced on [www.astm.org](http://www.astm.org).

<sup>4</sup> 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 be 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)**.<sup>5</sup>

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 amoeboid movement; there are several different types, classified into the two large groups granular leukocytes (basophils, eosinophils, and neutrophils) and nongranular leukocytes (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

<sup>5</sup> 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  $\pm$  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 holds 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 *Erythrocyte 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