

# Guidance for Industry

## For Platelet Testing and Evaluation of Platelet Substitute Products

### *DRAFT GUIDANCE*

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## **GUIDANCE FOR INDUSTRY<sup>1</sup>**

### **For Platelet Testing and Evaluation of Platelet Substitute Products**

#### **I. INTRODUCTION**

The original platelet testing guidelines were issued by the Food and Drug Administration (FDA) in July 1981 as announced in the Federal Register of October 2, 1981 (46 FR 48768). Since then, new instrumentation, such as flow cytometry, and new information about platelet physiology and biochemistry have altered the way that platelets can be evaluated. These advances have prompted the release of updated guidance regarding platelet testing and evaluation of platelet substitute products. This draft guidance, when finalized, will update and replace the June 1981 guideline entitled “Platelet Testing Guidelines” and delineate principles of general applicability for evaluation of platelets collected and processed by novel technologies.

#### **II. BACKGROUND**

Platelets participate in a number of reactions which contribute to maintaining thrombotic hemostasis in circulation. When activated by exposed subendothelium or by circulating agonists, platelets form aggregates which are incorporated into a platelet plug that prevents local hemorrhage. In addition, platelets also recruit neutrophils and monocytes by exposing P-selectin on their surface, contribute to signal transduction in neutrophils and endothelial cells by trans-cellular metabolism of released lipid precursors, serve as a site for activated clotting factor assembly and exert a physical force to retract clots. Together these diverse aspects of platelet physiology make up the clinical efficacy of platelets (1, 2).

The current gold standard of clinical platelet efficacy evaluation is in vivo survival of transfused radiolabelled platelets. It is based on the assumption that viable circulating platelets can participate in the responses which make up the clinical platelet efficacy. Because in vivo testing of radiolabelled platelets is associated with a small amount of risk, in vitro tests have been utilized as a screening process to eliminate procedures which clearly result in suboptimal platelet products (e.g., cold-stored platelet concentrates) without requiring human trials. Over the years no single in vitro test has stood out as a direct surrogate for platelet efficacy. It has now been recognized that a battery of tests, which examine different parts of platelet physiology, can produce a reasonable estimate of platelet efficacy in vivo.

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<sup>1</sup> This guidance document represents FDA’s current thinking on platelet testing and evaluation of platelet substitute products. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

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Many recent technological advances have been applied to the production and storage of platelet concentrates. The effects of these novel procedures on platelets should be evaluated against accepted conventional methods used in blood banking practice. The following tests are a guideline for evaluating platelets subjected to novel methodologies. Although it may not be feasible or appropriate to conduct all tests prior to a clinical evaluation (i.e., prior to submission of an Investigational New Drug Application (IND)[21 CFR 312]), a reasonable number of tests that look at different aspects of platelet physiology is desirable. The evaluation of platelets and platelet substitutes is grouped into four categories:

- A. In vitro Evaluation of Platelet Biochemistry and Function
- B. Platelet Survival in Circulation
- C. Clinical Hemostatic Efficacy
- D. Evaluation of Platelet Substitutes

### **III. SPECIFIC RECOMMENDATIONS**

#### **A. In vitro Evaluation of Platelet Biochemistry and Function**

The objective is to demonstrate that platelets which have been processed through a new collection procedure or subjected to new storage conditions can respond to a variety of stimuli equally well as platelets processed and stored by FDA approved blood banking practices. Many of these tests are further discussed and referenced in a review by the Biomedical Excellence for Safer Transfusion (BEST) task force (3).

*The state of platelets before and after procedure:*

*Morphology:*

Platelet morphology should be visually inspected at different levels of resolution, starting with a discs vs. spheres estimate (4, 5). The presence of different morphological forms should be quantitated (6), and finally the platelets should also be examined by electron microscopy (7).

*Biochemical status:*

For stored platelets, in vitro tests of platelets have generally not correlated well with platelet performance in vivo, however, platelet cellular levels of ATP, glucose, and lactate should offer some indication of platelet performance (3). A drop in platelet count with an increase in the level of lactate dehydrogenase in medium can be used as

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a measure of cellular lysis. The pH of platelet suspension above 7.6 and below 6.2 at the end of the storage period has been shown to correlate with decreased in vivo performance (3, 8).

*Markers to determine % of platelets activated by procedure:*

Activation of platelets is associated with surface expression of the following surface antigens: GMP-140 (P-selectin, CD 62), CD 63, and the active form (fibrinogen-binding) of GPIIb/IIIa (detected by PAC-1) (9).  $\beta$ -thromboglobulin and/or Platelet Factor 4 released by activated platelets into the medium are platelet-specific proteins and can be measured as indicators of platelet activation (10). Platelet Factor 3 activity (procoagulant surface for binding clotting proteins) also becomes increased with platelet activation (11).

*Physiologic responses:*

The functional ability of a platelet can be estimated by their response to osmotic stress and by the extent of agonist-induced shape change (12). Aggregation to increasing concentrations of physiologic agonists such as ADP, collagen, epinephrine, or to dual agonist combinations of ADP/epinephrine and ADP/collagen will give an idea of the responsiveness of the platelet (10). The presence the platelet serotonin uptake and agonist-induced serotonin secretion and agonist-induced expression of platelet activation markers such as GMP-140, will also evaluate the platelet physiologic responses (3).

*Quantitation of microparticles:*

The physiologic role of microparticles is not clear yet but they may be involved in thrombosis and/or immunogenic sensitization against platelets (13). Their quantitation will better characterize the platelet product.

**Comments:**

These tests should be run as a paired comparison with platelets stored in FDA approved storage containers (i.e., stored in gas permeable plastic bags, containing equal volumes, equal numbers of platelets and of white cells as the test platelets, on a rotator at 20-24° C). If an alternative storage medium other than plasma is used, the in vitro test conditions should mimic the conditions encountered by the platelets infused in vivo (i.e., resuspension in normal plasma). If non-plasma stored platelets are resuspended in plasma for in vitro testing and compared to plasma-stored platelets, the resuspending plasma should be equivalent to the plasma used for storage. That is, platelets resuspended in fresh-frozen plasma should not be compared to platelets resuspended in plasma stored at room temperature for 5 days.

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**B. Platelet Survival in Circulation**

Prolonged in vivo circulation survival of transfused platelets has been taken as a sign of a functional, undamaged platelet. Any new procedure for platelet collection and storage which does not demonstrate significant changes in platelet responses on in vitro tests should be further tested for its effects on platelet in vivo survival. Recommendations on carrying out such tests have been published (14). Design of such tests should include normal volunteers receiving radiolabelled autologous platelets subjected to the novel treatment. Recent advances in double labeling of platelets with <sup>111</sup>Indium and <sup>51</sup>Chromium for a simultaneous comparison in a single recipient of novel vs. conventional methods of platelet treatment have provided satisfactory results with less scatter in data points. The extent of data scatter will determine the number of volunteers that need to be tested to derive a clear conclusion about the effects of the test treatment. Filing of an IND [21 CFR 312] or an IDE [21 CFR 812] as appropriate is required before such studies are conducted.

**C. Clinical Hemostatic Efficacy**

Platelet efficacy in vivo has proven to be very difficult to define. Currently there are no adequate clinical tests which will demonstrate platelet efficacy. In the past, bleeding time was thought of as the test of platelet efficacy, but numerous published studies have demonstrated the lack of correlation time between surgical bleeding and skin bleeding time and the variability of the bleeding time even with a single patient (15).

Current surrogate end points for platelet efficacy have been a sufficient number of platelets (often taken as >20,000/ul) that have demonstrated a normal response to a battery of in vitro tests and normal in vivo half life. The assumption is that a sufficient number of circulating and intact platelets will offer adequate protection against spurious intraorgan bleeding (16, 17). Thus, the clinical performance (efficacy) of platelets obtained with novel methodologies should be evaluated by inclusion of these platelet products in clinical practice. Records of the hemostatic effectiveness, clinically defined by changes in epistaxis, hematuria, and/or petechia etc. should be included as a part of the IND application. Bleeding times may be submitted as additional data on efficacy of tested platelets.

**D. Evaluation of Platelet Substitutes**

Manufactured platelet substitutes have recently been introduced as an optional replacement for collected and stored human platelets. These substitutes often emulate a single aspect of hemostatic platelet response and thus have been even more difficult to define in terms of platelet efficacy based on in vitro tests and in vivo survival. For example, many platelet substitutes have a very short circulating half-life in vivo which

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would place them in the poor platelet efficacy category. Yet, the platelet substitutes can decrease bleeding time in a thrombocytopenic animal model (18). Thus, platelet substitutes may be able to replace one part of the platelet response and be used in specific clinical situations, such as acute trauma, as opposed to long term prophylaxis. The aspect of platelet response that the platelet substitute can replace should be defined and the clinical benefit to the recipient tested accordingly. In some instances, experienced investigators using a population of clinically stable aplastic thrombocytopenic patients have been able to correlate platelet count with skin bleeding time (19) or with stool blood loss using radiolabelled red blood cells (20). These studies suggest that platelet substitute efficacy could be demonstrated in a similar patient population using such methodology. Furthermore, an additional set of *in vitro* animal tests may be necessary for definition of efficacy and safety of platelet substitutes. These should include an evaluation of the following aspects:

#### *Evaluation of prothrombotic potential:*

A thrombosis model(s) in normal animals (21, 22) and in animals with disseminated intravascular coagulation (DIC) should be used for testing, since these conditions will be encountered clinically.

#### *Evaluation of immunogenicity:*

Infusion of only subcellular parts of the platelet may induce an immunogenic response. It should be demonstrated in test animals that the platelet substitute is no more immunogenic than intact platelets.

#### *Additional toxicity due to platelet additives:*

Chemicals that are used to produce platelet substitutes and remain in the final product should be evaluated for toxicity, mutagenicity, and carcinogenicity at plasma concentrations expected to be reached in a recipient of multiple platelet substitute transfusions, as could occur with a platelet-refractory patient. It should also be recognized that platelet substitutes, like platelets, will be given to recipients in their reproductive years and that reproductive toxicology and teratology studies on the additives may also need to be done. It should also be noted whether the additives interfere, such as by adding color to plasma, with common clinical laboratory test determinations.

#### **Comments:**

FDA has approved a number of current platelet products, collected and stored by accepted blood banking practices, as safe and effective therapies for thrombocytopenia. Platelet substitutes which aim to be alternatives to the current platelet product should demonstrate a clear benefit-to-risk ratio before they are considered for clinical trials.

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