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(54) Titre : PROCEDES POUR REDUIRE LA FORMATION DE CAILLOTS DANS DES CONCENTRES DE PLAQUETTES CONSERVES EN CHAMBRE FRIGORIFIQUE

(54) Title: METHODS TO REDUCE CLOT FORMATION IN COLD-STORED PLATELET PRODUCTS

(57) Abrégé/Abstract:
Methods to significantly reduce clot formation in cold-stored platelet samples are described. The methods include collecting platelet samples at defined yields and/or concentrations and allowing collected platelet samples to rest at room temperature without agitation for a period of time before being moved into cold storage.

ABSTRACT

Methods to significantly reduce clot formation in cold-stored platelet samples are described. The methods include collecting platelet samples at defined yields and/or concentrations and allowing collected platelet samples to rest at room temperature without agitation for a period of time before being moved into cold storage.

METHODS TO REDUCE CLOT FORMATION IN COLD-STORED PLATELET PRODUCTS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under grant W81XWH-12-0281 awarded by the Department of Defense. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0002] The current disclosure provides methods to significantly reduce clot formation in cold-stored platelet samples. The methods include collecting platelet samples at defined yields and/or concentrations and allowing collected platelet samples to rest at room temperature without agitation for a period of time before being moved into cold storage.

BACKGROUND OF THE DISCLOSURE

[0003] Whole blood contains various cellular components, such as red blood cells (RBC), platelets and white blood cells, suspended in a liquid plasma component. Donations of whole blood often are separated into the individual, clinically therapeutic components for individual storage and use in treating medical conditions that require administration of one or more particular blood component to a patient.

[0004] Platelets are non-nucleated bone marrow-derived blood cells that protect injured mammals from blood loss by adhering to sites of vascular injury and by promoting the formation of plasma fibrin clots. Humans depleted of circulating platelets by bone marrow failure suffer from life threatening spontaneous bleeding, and less severe deficiencies of platelets contribute to bleeding complications following trauma or surgery.

[0005] As the count of circulating platelets falls, patients become increasingly susceptible to cutaneous bleeding. Patients with platelet counts of less than 10,000 per μL are highly susceptible to spontaneous hemorrhage, especially when the low platelet count is caused by a bone marrow disorder or failure (e.g., aplastic anemia, acute and chronic leukemia, metastatic cancer, and deficiencies resulting from cancer treatment such as ionizing radiation or chemotherapy).

[0006] A major advance in medical care half a century ago was the development of platelet transfusions to correct platelet deficiencies. Currently, there are an estimated 2.6 million platelet transfusions in the United States per year.

[0007] Platelets for clinical use are currently stored at room temperature. Room temperature storage is based on discoveries made during the 1960s that cold-storage (e.g., at refrigerated temperatures) leads to a significant reduction in platelet survival in recipients.

[0008] The need to keep platelets at room temperature prior to transfusion has imposed a unique

set of costly and complex logistical requirements on platelet storage. Because platelets are metabolically active at room temperature, they require constant agitation in gas permeable containers to allow for the exchange of gases to prevent the toxic consequences of metabolic acidosis. Room temperature storage conditions result in macromolecular degradation and reduced hemostatic activity. Hemostatic activity broadly refers to the ability of a population of platelets to mediate bleeding cessation. The observed macromolecular degradation and reduced hemostatic activity of stored platelets are collectively referred to as the "platelet storage lesion" (PSL). In addition, storage at room temperature encourages the growth of bacteria. In this regard, bacterial contamination of platelets is by far the most frequent infectious complication of blood component use. At current rates, from one in 1,000 to one in 2,000 units of platelets are contaminated with bacteria at a level sufficient to pose a significant risk to the recipient.

[0009] Platelet storage at refrigerated temperatures has potential benefits, including better hemostatic function and a lowered risk of infectious diseases. Based on this, FDA-approval for the cold-storage of platelets has been sought. Stubbs et al., Transfusion, 57, 2836-2844 (Dec. 2017). However, in this study, while various benefits related to cold-storage were achieved, major challenges were noted including a high degree of product wastage. One reason for the high wastage included macro-aggregate clot formation within the stored samples. More particularly, macro-aggregate clot formation was observed in 18.2% of samples. Stubbs et al. concluded, "[i]f these limitations could be addressed, [cold-stored platelets] would become a much more practical option for bleeding patients." Page 2842, 2nd column. Stubbs et al., also stated that cold-stored platelets "with a shelf-life of 10 or more days would allow an expanded inventory of the blood product, and it could very well become the component of choice for all actively bleeding patients." Page 2843, 1st column.

SUMMARY OF THE DISCLOSURE

[0010] The current disclosure provides methods for cold-storage of platelet samples with significantly reduced clot formation. In fact, the methods disclosed herein reduced macro-aggregate clot formation from the 18.2% observed in Stubbs et al., Transfusion, 57, 2836-2844 (Dec. 2017) down to less than 3%. This significant decrease allows for better storage of platelet samples, allowing a much-needed expansion of the inventory of this important blood product.

[0011] In particular embodiments, the methods include one or more of the following characteristics: (i) collecting the platelets within plasma; (ii) collecting the platelets within a defined yield/bag; (iii) calibrating the concentration of platelets within plasma; (iv) allowing the collected sample to rest at room temperature before cooling; and/or (v) allowing the collected sample to

rest at room temperature without agitation before cooling.

[0012] In particular embodiments, platelets are collected by apheresis.

[0013] In particular embodiments, the defined yield/bag is $2.5X10^{11} – 4.5X10^{11}$ platelets/bag. In particular embodiments, the defined yield/bag is $3.0 X10^{11} – 4.0X10^{11}$ platelets/bag.

[0014] In particular embodiments, the concentration of platelets within plasma is calibrated to $0.5X10^{6} – 2.3X10^{6}$ platelets/μL plasma. In particular embodiments, the concentration of platelets within plasma is calibrated to $0.7X10^{6} – 2.1X10^{6}$ platelets/μL plasma. In particular embodiments, the concentration of platelets within plasma is calibrated to $1.5X10^{6}$ platelets/μL plasma.

[0015] In particular embodiments, platelet samples remain at room temperature for 6 hours or less before cooling to 4±2°C. In particular embodiments, platelet samples remain at room temperature for 4 hours or less before cooling to 4±2°C. In particular embodiments, platelet samples remain at room temperature for 2 hours or less before cooling to 4±2°C. In particular embodiments, platelet samples remain at room temperature for 1 hour before cooling to 4±2°C.

[0016] In particular embodiments, platelet samples remain at room temperature for 6 hours or less without agitation, for 4 hours or less without agitation, for 2 hours or less without agitation, or for 1 hour without agitation before cooling to 4±2°C.

[0017] In particular embodiments, platelets are (i) collected into plasma utilizing apheresis; (ii) collected into a bag with a yield of $3.0 X10^{11} – 4.0X10^{11}$ platelets/bag; (iii) calibrated to a concentration of $0.7X10^{6} – 2.1X10^{6}$ platelets/μL plasma; and (iv) allowed to rest at room temperature for 1 hour without agitation before being transferred to cold storage at 4±2°C without agitation.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0018] FIGs. 1A, 1B: Platelet Yields of Apheresis Units: Platelets were stored in either plasma alone (Plasma, black circles data were 3 day control units collected during 10 day plasma stored studies and downward triangles data were from 3 day control units collected during 15 day plasma stored studies), or in a 65% platelet additive solution (PAS), 35% plasma mixture (Intersol, black squares, or Isoplate, black, upward triangles). [Total platelet yield in the component, calculated by multiplying the platelet count of the sample times the volume of the component (platelet count x component volume = actual platelet yield)]: (1A) Platelets were stored for 3 days at 4°C. Results are shown as percentage of day 1 platelet counts. No significant differences were seen between the groups. (1B) 10 day and 15 day cold-stored platelets (CSP; as indicated) as percentage of the same subject's 3 day CSP. ns= not significant,**p<0.005.

[0019] FIGs. 2A-2E: In vitro Platelet Measurements: Platelets were stored in either plasma alone

[Plasma, black circles (10 day storage) and downward triangles (15 day storage)], or in a 65% PAS, 35% plasma mixture (Intersol, black squares, or Isoplate, black, upward triangles) at 4ºC, for either 10 or 15 days as indicated. (2A) glucose and (2B) lactate determined by blood gas analyses, (2C) Annexin V positive events, (2D) CD62P-positive (P-selectin) events, (2E) CD61-positive microparticles, determined by flow cytometry. All results are shown as percentage of the same subject's 3 day 4ºC-stored platelets. *p<0.05, **p<0.01, ***p<0.0001, ns= not significant.

[0020] FIGs. 3A, 3B: In Vivo Platelet Measurements: Healthy human subjects received their autologous radiolabeled platelets after storage at 4ºC. [Plasma, black circles (10 day storage) and downward triangles (15 day storage)], or in a 65% PAS, 35% plasma mixture (Intersol, black squares, or Isoplate, black, upward triangles). (3A) Recovery of transfused platelets at 1 hour time point. (3B) Survival of transfused platelets. All results given as percentage of the subject's 3 day autologous radiolabeled 4ºC-stored control platelets. *p<0.05, **p<0.01, ***p=0.001, ns= not significant.

[0021] FIG. 4: Platelet-integrin activation: 4ºC-stored platelets were stored for 10 days and either left unstimulated (baseline, left panel) or stimulated with 10uM ADP (final concentration) (ADP, right panel). The activation-dependent αIIbβ3-integrin antibody PAC-1 was incubated with both samples along with a activation independent β3-chain antibody (Y-axis, stains all platelets).

DETAILED DESCRIPTION

[0022] Platelets play a key role in hemostasis, clot stability and retraction, as well as in vascular repair and anti-microbial host defense. Thrombocytopenia, or low blood platelet count, can result from a number of conditions, which depending on severity, may require the transfusion of donor platelets for treatment. Platelet transfusions are particularly crucial in the treatment of patients with cancer or massive trauma.

[0023] Current clinical practice has platelet samples stored at room temperature (i.e., 20 to 24°C) after preparation. The requirement to store platelet samples at room temperature, however, limits the lifetime of the samples to 5 days, primarily due to concerns regarding bacterial contamination. In fact, bacterial contamination of platelet products for transfusion is a major safety problem in blood banking.

[0024] Different technologies have been developed aiming to minimize the risk of bacterial contamination. Examples include diversion pouches for collection, bacterial detection with automatic culture systems and pathogen reduction systems. While these advancements have reduced the number of cases of platelet transfusion associated sepsis, the risk has not been satisfactorily overcome.

[0025] Moreover, the current short shelf-life of platelet samples makes it difficult to maintain required inventories. At any given time, up to 20% of platelet samples can be wasted due to expiration. Thus, the extension of platelet sample shelf-life would strengthen the inventory of platelets available for clinical use.

[0026] Storage of platelets in cold temperatures would reduce the proliferation of most bacteria and allow a longer period of storage, minimizing shortages currently caused by the short storage time (5-day) approved by the FDA. Based on this, FDA-approval for the cold-storage of platelets has been sought. Stubbs et al., Transfusion, 57, 2836-2844 (Dec. 2017). However, in this study, while various benefits related to cold-storage were achieved, major challenges were noted including a high degree of product wastage. One reason for the high wastage included a short 3 day storage period before product expiration. A second major challenge included macro-aggregate clot formation in 18.2% of samples. Stubbs et al. concluded, "[i]f these limitations could be addressed, [cold-stored platelets] would become a much more practical option for bleeding patients." Page 2842, 2nd column. Stubbs et al., also stated that cold-stored platelets "with a shelf-life of 10 or more days would allow an expanded inventory of the blood product, and it could very well become the component of choice for all actively bleeding patients." Page 2843, 1st column.

[0027] The current disclosure provides methods for cold-storage of platelet samples with significantly reduced clot formation. In fact, the methods disclosed herein reduced macro-aggregate clot formation from the 18.2% observed in Stubbs et al., Transfusion, 57, 2836-2844 (Dec. 2017) down to less than 3%. This significant decrease allows for better storage of platelet samples, allowing a much-needed expansion of the inventory of this important blood product.

[0028] In one example of the disclosure, platelets are collected with a TRIMA® (Terumo BCT, Inc., Lakewood, CO) apheresis machine (Terumo BCT, Denver CO) using the licensed collection kit and ELP storage bags (Terumo BCT). Collections are performed with a yield of $4x10^{11}$/bag and a targeted concentration of $1.5x10^{6}$/µL. Because the actual concentration can differ from the targeted concentration, the concentration can be adjusted with the concurrently collected plasma to $1.5x10^{6}$/µL. The bag rests at room temperature for 1 hour before being transferred to the cold (4°±2C). The bag rests at room temperature without agitation and is only removed for sampling purposes. Using this way of storing the platelets, only one unit with a macro-aggregate clot was found 1/51=2%. The n=51 include varying time points between 3 days and 20 days of storage. As indicated, other groups have reported wastage rates of close to 20% with 3 days storage (Stubbs et al., Transfusion, 57, 2836-2844 (Dec. 2017)). Finally, a drop in platelet count was routinely observed, has been reported by other groups before, and likely

represents micro-aggregate formation (Getz et al., Transfusion. 2016;56(6):1320-8; Example 1).

[0029] In a second example of the disclosure, prior to apheresis, the pre-apheresis health history questionnaire and check of vital signs are completed. The subject's platelets are collected using the TRIMA ACCEL® (Terumo BCT, Inc., Lakewood, CO) Automated Blood Collection System which is licensed by the FDA for this purpose. A venipuncture site is selected and cleaned using standard procedures. A needle is placed in one of the subject's arms at the antecubital area. A complete blood count (CBC) sample is obtained using an inline diversion pouch. Whole blood is drawn into the apheresis machine and the blood components are separated by centrifugation. Platelets and plasma are collected into Terumo ELP storage bags and the red blood cells (RBC) are returned to the subject. Along with the return of the subject's RBC the subject receives 350 mL of ACD (citrate) anticoagulant during the collection process. The platelet apheresis collection lasts 2 hours. Subjects are observed throughout the collection by a nurse or technician specifically trained in apheresis.

[0030] A standard single apheresis platelet unit (target platelet yield 3.0 x 10^{11}/unit and concentration of 1500 x 10^3 platelets/μL) is collected. Fifty milliliters of concurrent plasma is also collected.

[0031] Units are calibrated to achieve a final platelet concentration of 0.7 – 2.1 x 10^6 platelets/μL, as per allowable bag parameters.

[0032] Immediately after apheresis collection and calibration using sterile techniques, the platelets are left in the attendant plasma. The units rest for 1 hour at room temperature prior to sampling for in vitro assays. Units are weighed to calculate platelet yield. The units are then placed in a locked cage in a refrigerator at 4±2°C and are not agitated during storage.

[0033] Temperature monitors record temperatures and trigger alarms for out of range conditions. End of storage is defined as the date and time when the aliquot is removed from the stored unit for infusion. To date, units have been cold-stored and tested up to 20 days.

[0034] Based on the foregoing examples of the disclosure, in particular embodiments, the methods include one or more of the following characteristics: (i) collecting platelets within plasma; (ii) collecting the platelets within a defined yield/bag; (iii) calibrating the concentration of platelets within plasma; (iv) allowing the collected sample to rest at room temperature before cooling; and/or (v) allowing the collected sample to rest at room temperature without agitation before cooling.

[0035] In particular embodiments, platelets are collected by apheresis.

[0036] In particular embodiments, the defined yield/bag is 2.5X10^{11} – 4.5X10^{11} platelets/bag. In

particular embodiments, the defined yield/bag is 3.0 $X10^{11}$ – $4.0X10^{11}$ platelets/bag.

[0037] In particular embodiments, the concentration of platelets within plasma is calibrated to $0.5X10^6$ – $2.3X10^6$ platelets/μL plasma. In particular embodiments, the concentration of platelets within plasma is calibrated to $0.7X10^6$ – $2.1X10^6$ platelets/μL plasma. In particular embodiments, the concentration of platelets within plasma is calibrated to $1.5X10^6$ platelets/μL plasma.

[0038] In particular embodiments, platelet samples remain at room temperature for 6 hours or less before cooling to 4±2°C. In particular embodiments, platelet samples remain at room temperature for 4 hours or less before cooling to 4±2°C. In particular embodiments, platelet samples remain at room temperature for 2 hours or less before cooling to 4±2°C. In particular embodiments, platelet samples remain at room temperature for 1 hour before cooling to 4±2°C.

[0039] In particular embodiments, platelet samples remain at room temperature for 6 hours or less without agitation, for 4 hours or less without agitation, for 2 hours or less without agitation, or for 1 hour without agitation before cooling to 4±2°C.

[0040] Particular embodiments include (i) collecting platelets collected into plasma utilizing apheresis; (ii) collecting platelets into a bag with a yield of 3.0 $X10^{11}$ – $4.0X10^{11}$ platelets/bag; (iii) calibrating the platelets to a concentration of $0.7X10^6$ – $2.1X10^6$ platelets/μL plasma; and (iv) allowing the collected and calibrated platelets to rest at room temperature for 1 hour without agitation before being transferred to cold storage at 4±2°C without agitation.

[0041] In particular embodiments, without agitation means that the samples (units) are not manually or mechanically manipulated for the purpose of letting the platelets rest after the apheresis collection which activates platelets due to shear stress. In particular embodiments, without agitation means that the samples are left on a surface free of manual or mechanical manipulation, but for what is required to test the samples for characteristics required for later use. In particular embodiments, without agitation means that the samples are left on a surface free of manual or mechanical manipulation, and without any manipulation or interference during the specified rest period (e.g., 1 hour).

[0042] Particular embodiments include populations of cold-stored platelet products produced according to the methods disclosed herein wherein 97% or more of the units within a population remain free of macro-aggregates for at least 10 days after cold storage begins. In particular embodiments, 97% or more of the units within a population remain free of macro-aggregates for at least 20 days after cold storage begins.

[0043] Particular embodiments include populations of cold-stored platelet products produced according to the methods disclosed herein wherein 85-100% of the units within a population remain free of macro-aggregates for at least 10 days after cold storage begins. In particular

embodiments, 85-100% of the units within a population remain free of macro-aggregates for at least 20 days after cold storage begins.

[0044] Particular embodiments include populations of cold-stored platelet products produced according to the methods disclosed herein wherein 85-97% of the units within a population remain free of macro-aggregates for at least 10 days after cold storage begins. In particular embodiments, 85-97% of the units within a population remain free of macro-aggregates for at least 20 days after cold storage begins.

[0045] Particular embodiments include populations of cold-stored platelet products produced according to the methods disclosed herein wherein 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the units within a population remain free of macro-aggregates for at least 10 days after cold storage begins. In particular embodiments, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the units within a population remain free of macro-aggregates for at least 20 days after cold storage begins.

[0046] In particular embodiments, free of macro-aggregates means that a cold-stored platelet product lacks visible macro-aggregate formation when visually examined by a blood-banking professional. In particular embodiments utilizing visual inspection, a macro-aggregate is a clot formation that is greater than 5 mm in size and exhibits an “egg drop soup appearance”. Units with such a macro-aggregate clot are not free of macro-aggregates and are not suitable for infusion.

[0047] In particular embodiments, free of macro-aggregates means that there are no aggregates greater than 2-3 mm by visual inspection, and that all observed aggregates resuspend or are eliminated in a first 200g spin in a radiolabeling procedure. If aggregates do not resuspend or are not eliminated in the first 200g spin, the unit is not free of macro-aggregates and is discarded.

[0048] Additional aspects and options of the disclosure are now described in the following additional detail:

[0049] Withdrawing blood from a donor typically includes inserting a needle into the donor's arm (and, more specifically, the donor's vein) and withdrawing blood from the donor through the needle. The "venipuncture" needle typically has attached to it one end of a plastic tube that provides a flow path for the blood. The other end of the plastic tube terminates in one or more pre-attached plastic blood containers (e.g., bags) for collecting the blood. The needle, tubing and containers make up a blood collection set which is pre-sterilized and disposed of after a single use. Conventional platelet bags or packs are formed of materials that are designed and constructed of a sufficiently permeable material to maximize gas transport into and out of the pack

(O_2 in and CO_2 out).

[0050] Single donor platelets are platelets obtained from one donor by means of centrifugal separation in an automated apheresis machine in a quantity sufficient to constitute one or more therapeutic dose(s) for subsequent transfusion to a patient(s). Platelets isolated by this method are generally known as single donor platelets because a therapeutic dose can be collected from a single donor. In such a procedure, the donor's blood flows from a point of venipuncture through a sterile centrifuge in which the platelets and a certain volume of plasma are centrifugally separated and isolated, with the balance of the donor's blood being returned to the donor through the initial venipuncture or a second point of venipuncture.

[0051] The blood collection container and tubing may include an anticoagulant. Anticoagulants can be used due to the tendency of blood to clot and adhere to the walls of surfaces, such as plastic surfaces. Exemplary anticoagulants are known in the art and include, an anticoagulant citrate phosphate dextrose (CPD) solution, an anticoagulant citrate phosphate double dextrose (CP2D) solution, an anticoagulant citrate phosphate dextrose adenine (CPDA) solution (e.g., CPDA-1), an acid citrate dextrose (ACD) solution (e.g., ACD-A), and an anticoagulant sodium citrate 4% w/v solution.

[0052] Various automated apheresis devices are commercially available from companies such as Terumo BCT (Lakewood, Colo.), Haemonetics Corporation (Braintree, Mass.), Fenwal, Inc. (Lake Zurich, Ill.), and Fresenius Kabi (Friedberg, Germany).

[0053] The collection of platelets by apheresis generally produces 2 platelet units, wherein each unit contains 200 to 300 mL of plasma and $3.5X10^{11}$ platelets. As indicated previously, and in particular embodiments, yields and concentrations according to the methods disclosed herein include $3.0\ X10^{11} – 4.0X10^{11}$ platelets/bag and $0.7X10^{6} – 2.1X10^{6}$ platelets/μL plasma.

[0054] While collection of platelets by apheresis is preferred, other methods may also be used. For example, the venipuncture method described above may be used to collect whole blood. It may be desirable for collection of whole blood to be completed relatively quickly, such as for example, with a whole blood donation time of less than 15 minutes. Whole blood collection volumes may vary, for example from 405-495 mL for 450 mL collection containers or from 450-550 mL for 500 mL collection containers. The sterile blood collection container typically serves as the primary container for initial separation of platelets in the buffy coat or platelet rich plasma (PRP) layer.

[0055] For preparation of platelets (e.g., whole-blood derived platelets) by the buffy coat method, whole blood is centrifuged under conditions to separate the components into a lower RBC layer, a middle buffy coat layer containing the platelets and an upper platelet poor plasma layer. Buffy

coat production methods for platelets are known in the art. Centrifugation conditions may be optimized according to blood center procedures, available centrifugation equipment, etc., but generally the initial centrifugation may be performed, for example, for 7 min. at 5000Xg at 22°C, or 5 min. The buffy coat is isolated, for example, by removing (e.g., expressing) the upper plasma layer and the lower red cell layer, leaving the buffy coat in the collection container. The isolated buffy coats may be further processed, for example, by adding plasma, by pooling (e.g., 4-6 buffy coat samples) and/or by adding a plasma additive solution (PAS) to achieve a desired volume and platelet concentration, followed by a lower speed "soft" centrifugation to separate the platelets from white blood cells. Such a lower speed centrifugation may be performed for example, for 3 min. at 2000Xg at 22°C or 8 min. at 500Xg at 22°C, followed by expressing the platelet suspension into a storage bag.

[0056] For preparation of platelets (e.g., whole blood-derived platelets) by the PRP method, whole blood is centrifuged under conditions to separate the components into a lower RBC layer containing white blood cells and an upper PRP layer. PRP production methods for platelets are known in the art. Centrifugation conditions may be optimized according to blood center procedures, available centrifugation equipment, etc., but generally the initial centrifugation is performed as a lower speed "soft spin", for example, for 3 min. at 2000Xg at 22°C. PRP is separated from the RBC layer by expressing the upper PRP layer (e.g., using a Compomat G-5), followed by a secondary, faster speed "hard" centrifugation of the PRP to separate the platelets from the plasma. Such a hard spin may be performed for example, for 5 min. at 5000Xg at 22°C, followed by removal of plasma from the platelet concentrate, leaving a desired volume of plasma for resuspension of the platelet component. The isolated PRP derived platelet components may be further processed, for example, by adding plasma, pooling (e.g., 4-6 samples) and/or adding a PAS to achieve a desired volume and platelet concentration.

[0057] At the time of collection and/or processing, blood may be identified or characterized with respect to one or more parameters, such as for example, hematocrit, hemoglobin, donor gender, whole blood volume, packed cell volume and/or platelet count.

[0058] The methods of the current disclosure can be used with platelets isolated by any technique known in the art or developed in the future so long as clotting of samples after 10 days does not exceed 3%.

[0059] The methods disclosed herein are utilized to make platelet samples which include platelet products and transfusion ready platelet products.

[0060] Platelet products are any blood derived product including platelets as the primary therapeutic component. Platelet products can further include blood plasma, anticoagulant solution

used during collection, and alternatively, or in addition, a suitable storage solution, such as a PAS.

[0061] Transfusion ready platelet products refer to a platelet product in a storage container (e.g., blood product bag) with suitable labeling for human use, which requires no further processing or treatment of the platelet contents prior to administration to a patient. In particular embodiments, such transfusion ready platelet products include platelets that have been subjected to a pathogen inactivation processing step (e.g., photochemical treatment, such as with a psoralen compound, to inactivate pathogens and leukocytes, if present) during preparation. A transfusion ready platelet product may be produced from an individual platelet unit or more than one unit (e.g., a pooled platelet product) and may be tested for one or more quality measures (e.g., bacterial testing, pH, platelet integrity assessed by platelet count and supernatant LDH, lactate concentration, platelet content, platelet morphology score, glucose concentration, platelet aggregation, extracellular adenosine triphosphate concentration, total adenosine triphosphate concentration, extent of shape change, and/or platelet hypotonic shock response).

[0062] PAS include any suitable aqueous composition that can be used in the storage of a platelet product. Such PAS typically provide nutrients and buffering capacity to allow for extended storage of platelets while maintaining suitable platelet function. PAS typically include sodium chloride and one or more components selected from citrate, phosphate, acetate, magnesium, potassium, calcium, gluconate, glucose, and bicarbonate. The following examples include sodium chloride and the indicated components: PAS-A (also referred to as PAS(1)) further including citrate, phosphate and potassium; PAS-B (also referred to as PAS II, PAS-2, SSP, or T-Sol) further including citrate and acetate: PAS-C (also referred to as PAS III, PAS-3, or Intersol) further including citrate, phosphate, and acetate; PAS-D (also referred to as Composol) further including citrate, phosphate, acetate, magnesium, potassium, and gluconate; PAS-E (also referred to as PAS IIIM or SSP+) further including citrate, phosphate, acetate, magnesium, and potassium; PAS-F (also referred to as PlasmaLyte A) further including acetate, magnesium, potassium, and gluconate; PAS-G further including citrate, phosphate, acetate, magnesium, potassium, and glucose; InterSol-G (also referred to as PAS IV) further including citrate, phosphate, acetate, magnesium, potassium, calcium and glucose; Isoplate (also referred to as Isolyte S) further including phosphate, acetate, magnesium, potassium, and gluconate; PAS V further including citrate, acetate, phosphate, magnesium, potassium, calcium, glucose, and bicarbonate; and M-Sol further including citrate, acetate, magnesium, potassium, calcium, glucose and bicarbonate.

[0063] A variety of suitable PAS may be used in the storage of platelets, where such solutions can be added to a unit of platelets in various amounts, such that a unit of platelets may include anywhere from, e.g., 0 to 95% PAS, 5 to 95% PAS, 50 to 95% PAS, 50 to 75% PAS. For example,

platelets may be stored in 65% PAS and 35% plasma, providing a unit of platelets including platelets, 65% PAS, and 35% plasma. Typically, in the methods described herein, a unit of platelets will be prepared to a desired level of plasma by addition of the PAS, either automatically in apheresis collection, or manually in the processing of buffy coat or PRP platelets. Platelet additives are described in terms of their aqueous concentration of components prior to their addition to the platelets to give the desired level of plasma in the additive containing unit of platelets.

[0064] As indicated, before administration to a patient as a transfusion ready platelet product, platelet sample function and characteristics can be assessed. Exemplary parameters for testing include platelet count, red and/or white blood cell amount (e.g., contamination), lipid contamination, platelet aggregation, platelet recovery, platelet viability, swirling pattern, potency, platelet survival, morphology, functional activity, activation markers, blood gas (pO2, pCO2), pH, glucose, lactate, volume, concentration of growth factors and icterus.

[0065] Contamination of platelets may be determined by any of several methods known in the art. For example, contamination of platelets with RBC can be determined by visual inspection for color indicative of RBC contamination. More specifically, RBC contamination above a certain level (e.g., >400 RBC/mL), results in platelets that exhibit discoloration from a light pink/salmon, reddish-orange color tinge to a marked red discoloration, which may be compared to standard visual inspection charts. Lipid contamination (e.g., lipemia) may similarly be determined by visual methods, with increased opacity, `milky` white appearance, large lipid particles that include lipoproteins and chylomicrons, and the like. Platelet aggregation may be determined visually and/or using any of a number of techniques and devices, such as for example, platelet aggregometry, optical aggregometers, lumi-aggregometers, light transmission aggregometry or turbidometric aggregometry. White blood cell contamination may be determined by counting, for example manually performing a leukocyte count (e.g., using a Neubauer counting chamber).

[0066] Platelet morphology may be visually inspected at different levels of resolution, including for example, with a discs vs. spheres estimate, and the presence of different morphological forms may be quantitated. Functionality can be estimated, for example, by platelet response to osmotic stress and by the extent of agonist-induced shape change. 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. Volume may be determined by weight and using a factor, such as 1.01 g/ml, as the specific gravity of platelets in PAS. 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 response. Additionally, platelet cellular levels of ATP, glucose, and lactate provide an indication of platelet performance. Activation of platelets is associated with surface expression of the various surface antigens, such as for example, GMP-140 (P-selectin, CD 62), CD 63, and the active form (fibrinogen-binding) of GPIIb/IIIa (detected by PAC-1). 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. Platelet Factor 3 activity (procoagulant surface for binding clotting proteins) also increases with platelet activation. Assays are also commercially available to perform such characterization of platelets in many cases.

[0067] The Exemplary Embodiments and Examples below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

[0068] Exemplary Embodiments.

1. A method of forming a cold-stored platelet product including:
   collecting platelet and plasma from a subject;
   calibrating the platelets to a concentration of $0.5X10^6$ – $2.3X10^6$ platelets/μL of the plasma to create a platelet sample;
   maintaining the platelet sample at room temperature for 6 hours or less, 4 hours or less or 2 hours or less; and
   transferring the platelet sample to cold-storage for a period of time, thereby forming a cold-stored platelet product.
2. A method of embodiment 1, wherein the collecting is by apheresis.
3. A method of embodiment 1 or 2, wherein the collecting of platelets is to a yield of $3.0\ X10^{11}$ – $4.0X10^{11}$ platelets/bag.
4. A method of any of embodiments 1-3, wherein the concentration is $0.7X10^6$ – $2.1X10^6$ platelets/μL of the plasma.
5. A method of any of embodiments 1-3, wherein the concentration is $1.5X10^6$ platelets/μL of the plasma.
6. A method of claim any of embodiments 1-5, wherein the maintaining the platelet sample at room temperature is for 1 hour.
7. A method of any of embodiments 1-6, wherein the maintaining the platelet sample at room temperature is without agitation of the platelet sample.
8. A method of any of embodiments 1-7, wherein room temperature is 20-24°C.

9. A method of any of embodiments 1-8, wherein the cold storage is 4±2°C.
10. A method of any of embodiments 1-8, wherein the cold storage is 4°C.
11. A method of any of embodiments 1-10, wherein the period of time is at least 24 hours.
12. A method of any of embodiments 1-10, wherein the period of time is 3-20 days.
13. A method of any of embodiments 1-10, wherein the period of time is 10 days or more.
14. A method of any of embodiments 1-13, wherein the cold-stored platelet product is free of macro-aggregates for up to 20 days.
15. A method of any of embodiments 1-14, wherein the platelet product is a transfusion ready platelet product.
16. A population of cold-stored platelet products produced according to any of the methods of embodiments 1-15 wherein 97% or more of the units within the population remain free of macro-aggregates for at least 10 days after cold storage begins.
17. A population of embodiment 16, wherein 97% or more of the units within the population remain free of macro-aggregates for at least 20 days after cold storage begins.
18. A population of cold-stored platelet products produced according to any of the methods of embodiments 1-15 wherein 85% - 97% of the units within the population remain free of macro-aggregates for at least 10 days after cold storage begins.
19. A population of embodiment 18, wherein 85% - 97% of the units within the population remain free of macro-aggregates for at least 20 days after cold storage begins.
20. A population of cold-stored platelet products produced according to any of the methods of embodiments 1-15 wherein 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the units within the population remain free of macro-aggregates for at least 10 days after cold storage begins.
21. A population of embodiment 20, wherein 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the units within the population remain free of macro-aggregates for at least 20 days after cold storage begins.
22. A population of any of embodiments 16-21, wherein the platelet products are transfusion ready platelet products.

[0069] Example 1. In vivo Viability of Extended 4°C Stored Autologous Apheresis Platelets. Introduction. Most U.S. Blood Banks store platelets at room temperature under gentle agitation with a maximum storage time of 5 days unless additional in vitro bacterial testing is done which permits 7 day storage. Previous studies demonstrated that storing platelets in the cold (4°C, CSP) resulted in a significant reduction in both in vivo recoveries and survivals compared with room temperature platelets (RSP) stored for the same times. Murphy & Gardner, N Engl J Med

1969;280: 1094-8. Subsequent studies with cold-stored platelets identified a clearance mechanism involving GPIb clustering on the platelet surface and in vivo binding to complement type 3 receptors with subsequent hepatocyte internalization. Hoffmeister et al., Cell 2003;112: 87-97; Snyder & Rinder, N Engl J Med 2003;348: 2032-3. In vitro studies suggest that 4°C-stored platelets have superior functionality compared with room temperature-stored platelets. Bynum et al., Transfusion 2016;56 Suppl 1: S76-84; Getz et al., Transfusion 2016;56: 1320-8; Nair et al., Br J Haematol 2017;178: 119-29; Reddoch et al., Shock 2014;41 Suppl 1: 54-61; Becker et al., Transfusion 1973;13: 61-8. Furthermore, storage of platelets in the cold (4°C) has the advantage of potentially prolonging storage times while reducing post-transfusion infections. Room-temperature storage has led to a 5 day storage limit since bacterial growth and septic reactions increase over time. Braine et al., Transfusion 1986;26: 391-3. This limited shelf-life leads to periodic shortages on the one hand and frequent outdates on the other. Contrary to red cells, platelet usage is still slightly increasing according to recently published data. Braine et al., Transfusion 1986;26: 391-3. Having an additional platelet inventory with extended storage in the cold for specifically targeted patient populations could lead to both greater platelet availability and fewer outdates.

[0070] When first introduced, platelet transfusions were predominantly used for the prophylactic transfusion support of hematology/oncology patients with hypoproliferative thrombocytopenia. These patients benefit from prolonged post-transfusion platelet survivals to decrease transfusion frequency. However, a recent analysis showed that as many as 50% of the platelets transfused are given to non-hematology/oncology patients (i.e., trauma, surgery, ICU, and general medicine patients) who often require only short-term hemostatic support. Braine et al., Transfusion 1986;26: 391-3.

[0071] CSP corrected the bleeding time quicker than RSP in thrombocytopenic patients. Becker et al., Transfusion 1973;13: 61-8. However, the data on in vivo efficacy of 4°C-stored platelets are not uniformly in favor of 4°C-storage. The majority of bleeding time measurements in thrombocytopenic patients transfused with cold-stored platelets remained markedly prolonged, while most measurements in the control group (stored at 22°C) improved. Slichter & Harker, Br J Haematol 1976;34: 403-19. In addition, Filip et al. found that 4°C stored platelets were less effective after 72h of storage when compared to 22°C-stored platelets. Filip & Aster, J Lab Clin Med 1978;91: 618-24. More studies are needed to conclusively settle the debate if cold-stored platelets function better than RSP in vivo.

[0072] Two randomized, controlled clinical trials are available suggesting that CSP are more hemostatically active than RSP. A trial in pediatric cardiac surgery patients showed that whole

blood stored in the cold (4ºC) was more effective in reducing blood loss during open heart surgery compared with reconstituted whole blood containing RSP (22ºC). Interestingly, patients who received cold-stored whole blood had significantly better aggregometry responses than RSP-containing reconstituted whole blood. Manno et al., Blood 1991;77: 930-6. Preliminary analysis of an ongoing Norwegian randomized, pilot-trial in open-heart surgery patients showed that patients who received up to 7 day stored CSP in a platelet additive solution (T-PAS, Terumo BCT, Denver, CO) had significantly reduced post-operative blood loss with CSP compared with RSP. Manno et al., Blood 1991;77: 930-6.

[0073] Currently, the FDA allows transfusion of 3 day CSP (whole-blood derived pooled platelets or apheresis platelets) for actively bleeding patients. Cap, Transfusion 2016;56: 13-6. CSP have not been widely utilized presumably due to a lack of data suggesting superior efficacy in the target population of bleeding patients. Furthermore, the 3 day storage limit is even shorter than the limit for RSP, thus offering even less flexibility.

[0074] Platelet storage in PAS has been shown to allow for prolongation of the storage time in room temperature platelet storage studies. Slichter et al., Blood 2014;123: 271-80. Currently, only two PAS are licensed by the FDA for room temperature storage, and they have not been widely adopted. Getz et al., Transfusion 2016;56: 1320-8; Manno et al., Blood 1991;77: 930-6; Slichter et al., Blood 2014;123: 271-80. It is unknown how PAS may affect in vivo viability of platelets stored at 4ºC. In the current study, the in vivo viability of autologous radiolabeled extended cold stored platelets in plasma versus PAS in normal subjects along with in vitro tests currently required by the FDA for platelet licensing were investigated.

[0075] Materials & Methods. Preparation of Control and Test platelets. A double hyperconcentrated apheresis platelet unit was collected from 20 healthy adult subjects using the TRIMA ACCEL® Automated Blood Collection System (TerumoBCT, Denver, CO). After collection, the unit was split into two equal portions, and one unit was re-suspended in 100% plasma for 3 day 4°C storage (control unit was stored for the longest FDA licensed time), and the other unit was stored for an extended period of time at 4°C (test unit). The test unit was suspended in 100% plasma or 35% plasma and 65% PAS (either Isoplate [PAS-F, TerumoBCT, Denver, CO] or Intersol [PAS-3, Fenwal Inc., Lake Zurich, IL]). Both control and test units had to achieve a final platelet concentration of 1500 x 10^3 platelets/µL and they were stored, without agitation, at 4°C. The test units were stored for up to 10 days (plasma or plasma/PAS) or 15 days (plasma).

[0076] Radiolabeling of stored platelets. Nineteen out of 20 subjects were available for in vivo assessment. One subject did not show for autologous transfusion. Platelets were radiolabeled as previously described, following the detailed Biomedical Excellence for Safer Transfusion (BEST)

collaborative protocol. (BEST)Collaborative. TBEfST. Platelet radiolabeling procedure. Transfusion 2006;46:59S-66S. In brief, Indium (In-111, Anazao, Tampa, FL, USA) was used to label both control and test platelets because according to earlier experiments (data not shown), the other available isotope, chromium, demonstrated very poor uptake of Cr-51 by refrigerated platelets. The In-111 administered on Day 3 CSP was largely undetectable by Day 10 and therefore, re-use of the same isotope to measure both control and test cold stored platelets was considered feasible. Additionally, a pre-infusion radioactivity sample was collected prior to the "test" transfusion to account for any possible residual In-111 activity after each subject's control transfusion. Calculations were adjusted accordingly.

[0077] On Day 3, the subject received an In-111 radiolabeled aliquot of their control CSP stored platelet unit. Follow-up samples from the subject were collected 2 hours post-infusion and on Days 1 (x2, 2-10 hours apart), 2, 3, 4, and 5 to calculate recovery and survival of the subject's 3 day stored platelets.

[0078] The CSP test platelets in 100% plasma or PAS/plasma were initially stored for 10 days, and another group of subjects had their platelets stored in 100% plasma for 15 days. After storage of their test units, an aliquot of their test units was obtained for In-111 labeling and subsequently transfused. Follow-up samples, as above, were collected to calculate platelet recovery and survival of the test platelets. These studies allowed direct comparisons of the same subject's control 3 day CSP versus 10- or 15 day test stored CSP. Comparisons were further facilitated as both control and test platelets were labeled with the same isotope.

[0079] In vitro platelet measurements. Platelet counts of collected products were performed on the day following collection to allow platelet disaggregation that might have occurred during collection and at the end of storage using an ABX Hematology Analyzer (ABX Diagnostics, Irvine, CA). Platelet yields were calculated by multiplying the platelet count times the volume of the platelet unit. After storage, in vitro measurements of glucose and lactate concentration, and pH at 4°C were measured with a commercially-available blood gas instrument (Radiometer Medical, ABL Flex 805, Copenhagen, Denmark). Annexin V binding, P-selectin expression, microparticle quantification and mean platelet volume (MPV) were performed by flow cytometry (FACSCalibur, Beckman Coulter, Indianapolis, IN, USA) as previously described. Kunicki et al., Transfusion 1975;15: 414-21. The following antibodies were utilized: P-Selectin CD62P-FITC (BD Biosciences, San Jose, CA, USA); GPIbα-PE (BD Biosciences, San Jose, CA, USA); and Annexin-V-FITC (BD Biosciences, San Jose, CA, USA).

[0080] Statistical analysis. Results are reported as mean±one standard deviation, and statistical significance was assessed by unpaired, 2-tailed Student t test, unless otherwise indicated. A P

value equal or less than 0.05 was considered significant.

[0081] Results. Total platelet yield after storage. Post-storage platelet counts of the "control" units averaged 3.57±0.38 x 10^{11} post-storage (99±3% of pre-storage values). No significant differences in platelet yields between PAS and plasma stored platelets were seen after 3 days of storage (FIG. 1A). For the "test" 10-day plasma, Intersol, and Isoplate, and 15 day plasma units, post-storage platelet counts averaged 2.86±0.39 x 10^{11}, 2.82±0.35 x 10^{11}, 2.52±1.28 x 10^{11}, and 2.40±0.36 x 10^{11}, respectively (80±7%, 107±12%, 90±39%, and 72±14 % of pre-storage values). A significant reduction after 10 day and 15 day storage in plasma when compared to 10 day PAS Intersol-stored platelets ($p=0.001$ and $p=0.002$, respectively) was found. Platelet yields of 10 day plasma-stored, Intersol-stored, and 15 day plasma-stored did not differ significantly when compared to 10 day Isoplate-stored platelets either absolute or as percentage of 3 day CSP (FIG. 1B). Overall, these studies demonstrate that storing platelets in plasma might have the disadvantage of losing platelets either to the wall of the bag or by micro-aggregation of platelets mediated by fibrinogen as described previously by Getz et al., Transfusion 2016;56: 1320-8 (FIG. 1B).

[0082] In vitro platelet measurements. As expected, glucose levels were significantly lower in either Intersol or Isoplate PAS compared to plasma stored units (FIG. 2A, 10 day plasma, Intersol, Isoplate, 15 day plasma: 321±61mg/dl, 99±9 mg/dl, 82±41mg/dl, 321±81mg/dl, [90±2%, 28±3%, 24±12%, 86±4% of day 3 storage values, respectively], $p<0.0001$ for Intersol and Isoplate versus 10 day plasma-stored units) and, inversely, lactate levels were significantly elevated in plasma stored units compared with either Intersol or Isoplate stored units (FIG. 2B, 10 day plasma, Intersol, Intersol, 15 day plasma: 8.1±1.3mmol/l, 4.4±0.7mmol/l, 4.0±1.9mmol/l, 9.2±1.5mmol/l, [184±11%, 137±26%, 119±54%, 222±10% of day 3 storage values, respectively] $p=0.003$ for 10 day plasma versus Intersol and $p=0.007$ for 10 day plasma versus Isoplate). Lactate was also significantly higher in plasma stored for 15 days compared to 10 days of plasma storage indicating ongoing metabolic activity in plasma (FIG. 2B, $p=0.012$). The removal of plasma (which contains glucose) during preparation of the PAS/plasma units was likely the reason for the lower amount of glucose.

[0083] Lower glucose was also likely the reason for the lower lactate, as removal of plasma with glucose deprived them of the essential energy source. It is also possible that platelets in PAS are less metabolically active compared with platelets stored in plasma, however, this will require further investigation. Markers indicating membrane orientation changes with phosphatidyl serine exposure (% Annexin V-binding, FIG. 2C) only showed a significant difference between Intersol and 15 day plasma ($p=0.034$) (FIG. 2C, 10 day plasma, Intersol, Isoplate, 15 day plasma:

16.5±11.7%, 16.1±5.1%, 15.8±3%, 26.5±12.4% [372±219%, 246±87%, 301±106%, 730±413% of day 3 storage values], respectively). There was a trend for lower degranulation as measured by platelet alpha granule secretion (P-selectin expression) with Intersol when compared with 10 day plasma which did not reach statistical significance (p=0.08), however when Intersol was compared to 15 day plasma a significant difference was detected (p=0.004). (FIG. 2D, 10 day plasma, Intersol, Isoplate, 15 day plasma: 31±28%, 21.8±38.6%, 48.6±21.5%, 40.2±15.3%, [203±76%, 123±53%, 267±118%, 278±102% of day 3 storage values], respectively). Microparticles were significantly elevated in 15 day plasma compared to 10 day plasma-stored platelets (p=0.03) (FIG. 2E, 10 day plasma, Intersol, Isoplate, 15 day plasma: 1.7x105±1.6x105/μL, 6.1x104±3.5x104/μL, 7.4x104±2x104/μL, 4.1x105±2.1x105/μL, [158±127%, 186±88%, 188±41%, 561±605% of day 3 storage values], respectively), but there was no significant difference between plasma and PAS solutions at 10 days of storage. For all platelet activation parameters there was either a trend or a significantly higher value in 15 day plasma-stored units compared with 10 day plasma-stored units indicating continuous in vitro activation of platelets in plasma.

[0084] In vivo platelet viability. For the 3 day "control" units (n=19), post-storage platelet recoveries averaged 43±11% and survivals 2±0.4 days. For the "test" 10 day plasma, Intersol, Isoplate, and 15 day plasma units, post-storage recoveries averaged 24±8%, 18±4%, 8±2%, and 11±3% respectively (FIG. 3A, 55±11%, 43±6%, 21±8%, and 29±3%, respectively of the same subject's 3 day control data). The recovery of 10 day Isoplate and 15 day-stored plasma platelets was found to be significantly lower compared with both 10 day plasma and Intersol-stored platelets (FIG. 3A, p=0.002 and p=0.004 for 10 day Isoplate versus 10 day plasma and Intersol respectively, as well as p=0.001 and p=0.002 for 15 day plasma versus 10 day plasma and Intersol respectively). Interestingly, there was no significant difference between the recoveries of 10 day Intersol and plasma stored platelets, although there was a trend for a lower recovery with Intersol (FIG. 3A, p=0.057). Notably, Isoplate-stored platelets showed significantly lower recoveries compared with Intersol-stored platelets (p=0.004). Post-transfusion survivals for the 10 day CSP stored in plasma, Intersol, Isoplate, and 15 days in plasma averaged 1.2±0.3 days, 1.1±0.3 days, 0.9±0.8 days, and 0.7±0.2 days respectively (FIG. 3B, 59±12%, 56±8%, 48±42%, and 37±7%, respectively of the same subject's 3 day data). Platelet in vivo survival studies showed a significantly lower survival with platelets stored in plasma for 15 days compared to any of the 10 day stored platelets (FIG. 3B, p=0.01), but there were no significant differences among the 10 day stored platelet groups.

[0085] Discussion. Example 1 provides four major findings to highlight: First, the platelet yield is

significantly lower in plasma compared with platelets stored in Intersol. Second, cold-stored platelets are metabolically active and consume glucose, produce lactate, and show signs of increasing pre-activation. Third, different PAS solutions can yield significantly different in vivo results which cannot be predicted by their in vitro results. In the present study, Isoplate showed a significantly lower recovery, and a significantly lower platelet yield compared with Intersol, even though both have in vitro parameters mostly in the same range. Fourth, platelet recovery appears to be better in plasma compared with PAS solutions, even though the platelet yield in vitro is significantly higher with Intersol compared with plasma.

[0086] In vivo survival is likely not a major factor for cold-stored platelets, since the current target patient population for use of these platelets are those with active bleeding, who are in need of platelets to facilitate immediate localized hemostasis. Platelet survivals did not differ among any of the 10 day CSP but was significantly less at 15 days.

[0087] Previous in vivo animal imaging studies suggest that hemostasis and local clot formation are processes which require minutes (3-20min) and are not accomplished within seconds. Stolla et al., Blood 2011;117: 1005-13. The one hour in vivo platelet recovery measurement is likely an important parameter for transfused platelets since, in order to be hemostatically active, some platelets need to circulate for at least 60 min. In addition, the time-frame required to complete major surgery itself is likely to require several hours. One of the major findings is that there are significant differences between PAS solutions for cold-storage. While platelets collected in Intersol showed recoveries which were not significantly different than plasma ($p=0.057$), Isoplate showed significantly lower recoveries ($p=0.002$). Previous studies suggest that platelet function is better preserved during cold-storage in PAS and that there is less platelet aggregate formation in PAS compared with plasma. Getz et al., Transfusion 2016;56: 1320-8. A lower platelet yield in plasma compared with PAS solutions was observed, but overall platelet recoveries were better in plasma compared with PAS. This either suggests that any potential aggregates which pass the bedside transfusion filter disaggregate in vivo and are of no clinical significance, or that the aggregates are filtered out by the transfusion filter and platelets that pass the bedside transfusion filter have a higher recovery compared with PAS stored platelets.

[0088] A recently presented randomized Norwegian pilot study of 20 patients in each arm showed overall less blood loss in cardiac surgery patients after chest closure with cold-stored platelets stored in the PAS T-PAS (Terumo BCT, Denver, CO) compared with RT-stored platelets. Manno et al., Blood 1991;77: 930-6. T-PAS was not investigated because it currently is not available in the US.

[0089] Better in vivo function measured by reduced blood loss in surgery patients has yet to be

demonstrated with plasma-stored CSP.

[0090] It is acknowledged that in the current studies, collection of platelets in Intersol and Isoplate was conducted on the TRIMA® system [TRIMA is licensed for use with Isoplate, while Intersol requires collection with the Fenwal Amicus system (Fresenius Kabi, Bad Homburg, Germany)]. It is not believed that this difference was a major determinant in the study.

[0091] Should cold-stored platelets be considered for transfusion support in massively bleeding patients, the additional plasma in the plasma-stored unit may be of importance. Recent studies have suggested that a higher plasma to red cell ratio (1:1:1; plasma, platelets, RBCs respectively) could be beneficial in trauma patents. Holcomb et al., JAMA 2015;313: 471-82. Platelet units in plasma are simpler to prepare than units which require replacement of plasma with PAS in a very specific ratio, a significant factor given that these platelets are currently targeted as a future primary choice for blood banks in remote locations and far forward military scenarios. Patients who are in need of functional platelets are frequently coagulopathic as well and could benefit from the additional plasma in the platelet unit, and the minor platelet loss due to microaggregates during storage in plasma may not outweigh the loss of plasma when opting for the replacement with PAS. It should also be emphasized that while one group reported visible aggregates in platelet units stored in plasma at 4ºC (Getz et al., Transfusion 2016;56: 1320-8), visible aggregates were not observed in the plasma stored units described herein. The only evidence for microaggregates in the disclosed study was based on the lower platelet yield in plasma versus PAS. However, data (not shown) suggests that this loss is in part due to loss of platelets that adhere to the bag walls.

[0092] In summary, Example 1 describes the first to compare recovery and survival of CSP in PAS and plasma in healthy human subjects. It was found that both 65% Intersol / 35% plasma and 100% plasma CSP have potential advantages over the other.

[0093] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms “include” or “including” should be interpreted to recite: “comprise, consist of, or consist essentially of.” The transition term “comprise” or “comprises” means includes, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase “consisting of” excludes any element, step, ingredient or component not specified. The transition phrase “consisting essentially of” limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically significant increase in clot formation of platelet samples 10 days

after collection.

[0094] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term "about" has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of±20% of the stated value;±19% of the stated value;±18% of the stated value;±17% of the stated value;±16% of the stated value;±15% of the stated value;±14% of the stated value;±13% of the stated value;±12% of the stated value;±11% of the stated value;±10% of the stated value;±9% of the stated value;±8% of the stated value;±7% of the stated value;±6% of the stated value;±5% of the stated value;±4% of the stated value;±3% of the stated value;±2% of the stated value; or±1% of the stated value.

[0095] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0096] The terms "a," "an," "the" and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element

essential to the practice of the invention.

[0097] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0098] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0099] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0100] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0101] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0102] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

CLAIMS

What is claimed is:

1. A method of forming a cold-stored platelet product comprising:
   collecting platelet and plasma from a subject;
   calibrating the platelets to a concentration of $0.5X10^6 – 2.3X10^6$ platelets/μL of the plasma to create a platelet sample;
   maintaining the platelet sample at room temperature for 2 hours or less; and
   transferring the platelet sample to cold-storage for a period of time, thereby forming a cold-stored platelet product.
2. A method of claim 1, wherein the collecting is by apheresis.
3. A method of claim 1, wherein the collecting of platelets is to a yield of $3.0 X10^{11} – 4.0X10^{11}$ platelets/bag.
4. A method of claim 1, wherein the concentration is $0.7X10^6 – 2.1X10^6$ platelets/μL of the plasma.
5. A method of claim 1, wherein the concentration is $1.5X10^6$ platelets/μL of the plasma.
6. A method of claim 1, wherein the maintaining the platelet sample at room temperature is for 1 hour.
7. A method of claim 1, wherein the maintaining the platelet sample at room temperature is without agitation of the platelet sample.
8. A method of claim 1, wherein room temperature is 20-24°C.
9. A method of claim 1, wherein the cold storage is 4±2°C.
10. A method of claim 1, wherein the cold storage is 4°C.
11. A method of claim 1, wherein the period of time is at least 24 hours.
12. A method of claim 1, wherein the period of time is 3-20 days.
13. A method of claim 1, wherein the period of time is 10 days or more.
14. A method of claim 1, wherein the cold-stored platelet product is clot-free for up to 20 days.
15. A method of claim 1, wherein the platelet product is a transfusion ready platelet product.
16. A population of cold-stored platelet products wherein 97% or more of the population remains free of macro-aggregates for at least 10 days after cold storage begins.
17. A population of claim 16, wherein 97% or more of the population remains free of macro-aggregates for at least 20 days after cold storage begins.
18. A population of claim 16, wherein the platelet products are transfusion ready platelet products.
19. A population of claim 16, formed by a method of any of claims 1-15.

FIG. 1A

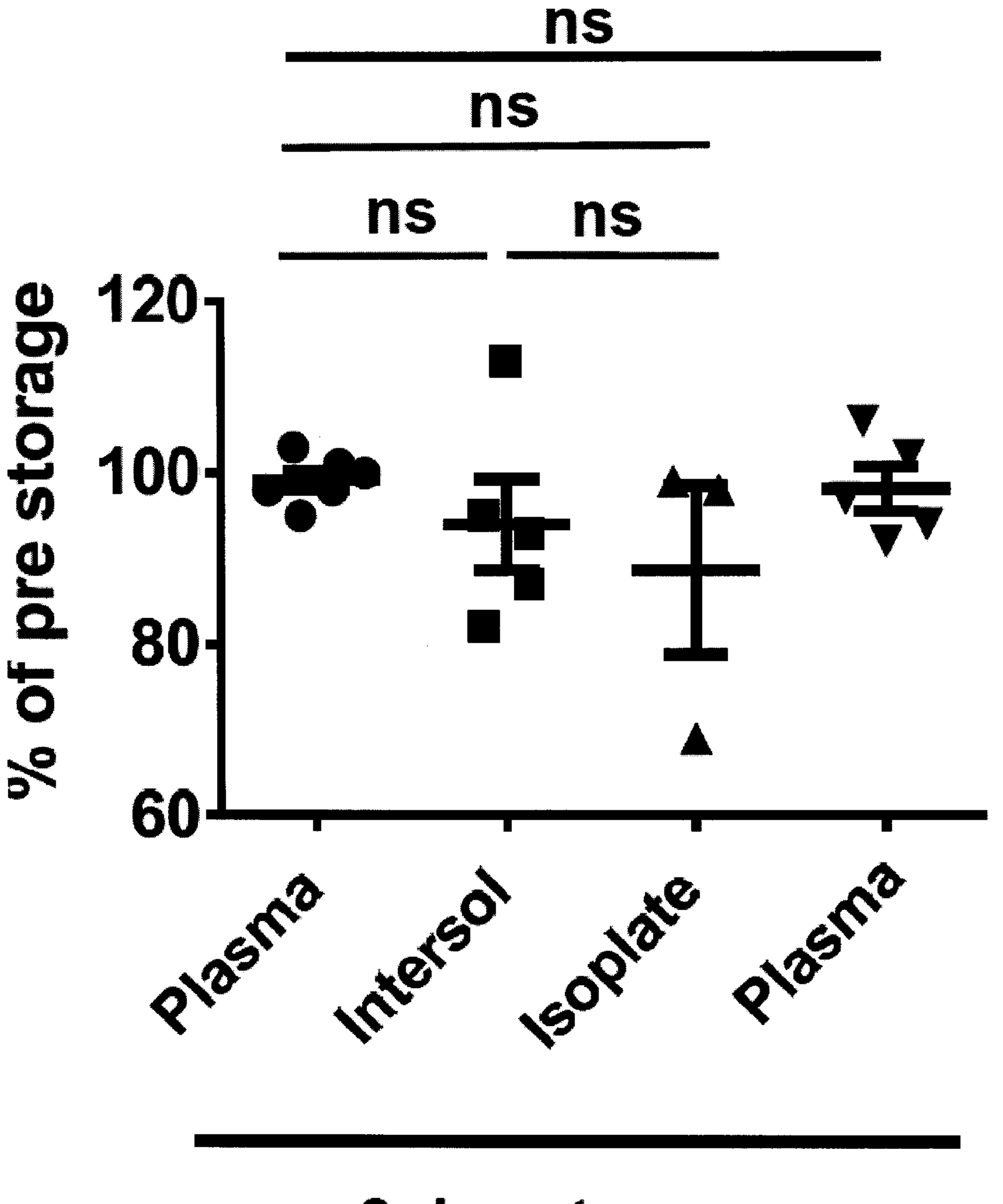

ns
ns
ns
ns
% of pre storage
120
100
80
60
Plasma
Intersol
Isoplate
Plasma
3 day storage

FIG. 1B

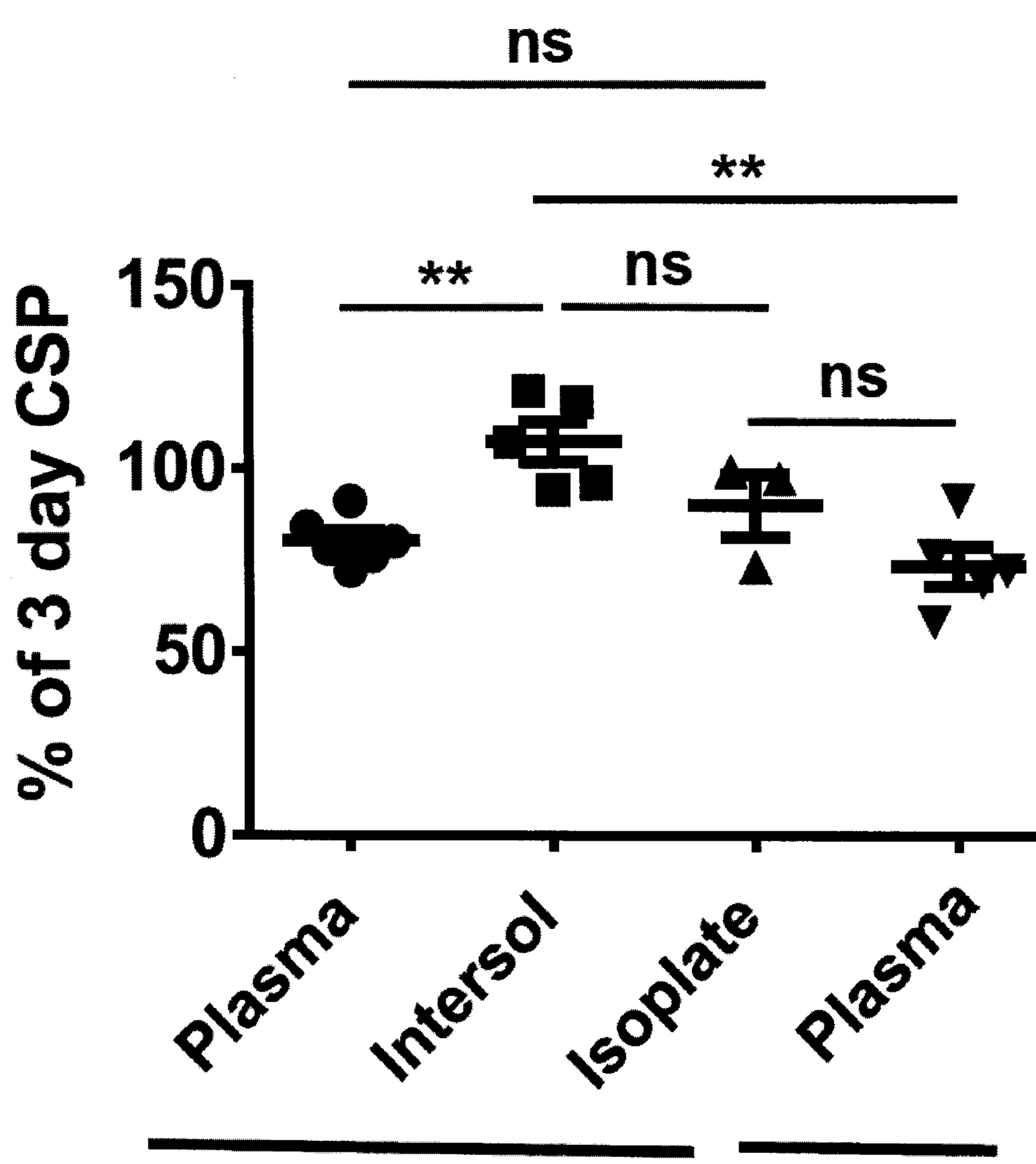

ns
**
**
ns
ns
150
100
50
0
% of 3 day CSP
Plasma
Intersol
Isoplate
Plasma
10 day storage
15 day storage

FIG. 2A

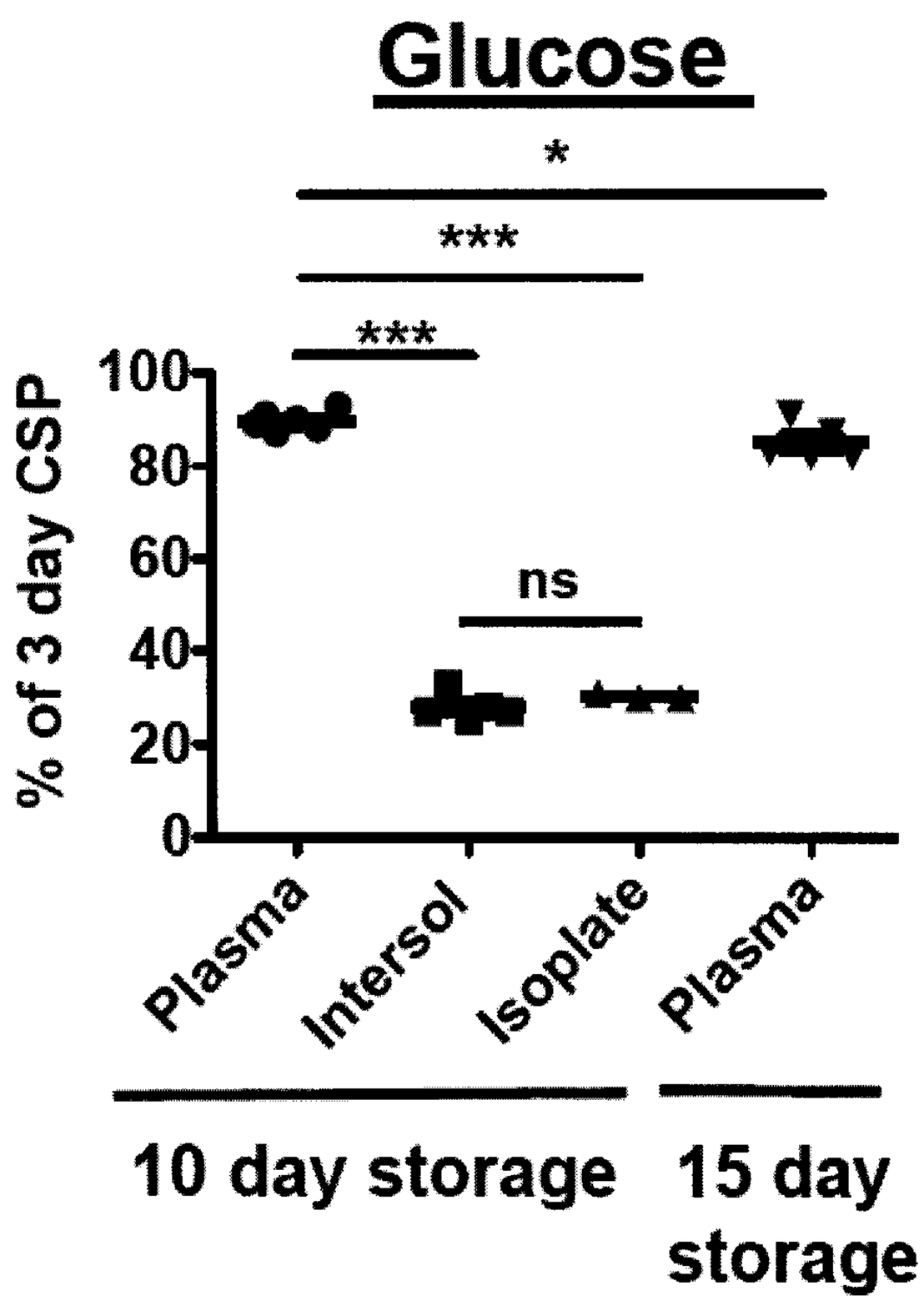

Glucose
*
***
***
100
80
60
40
20
0
% of 3 day CSP
ns
Plasma
Intersol
Isoplate
Plasma
10 day storage
15 day storage

FIG. 2B

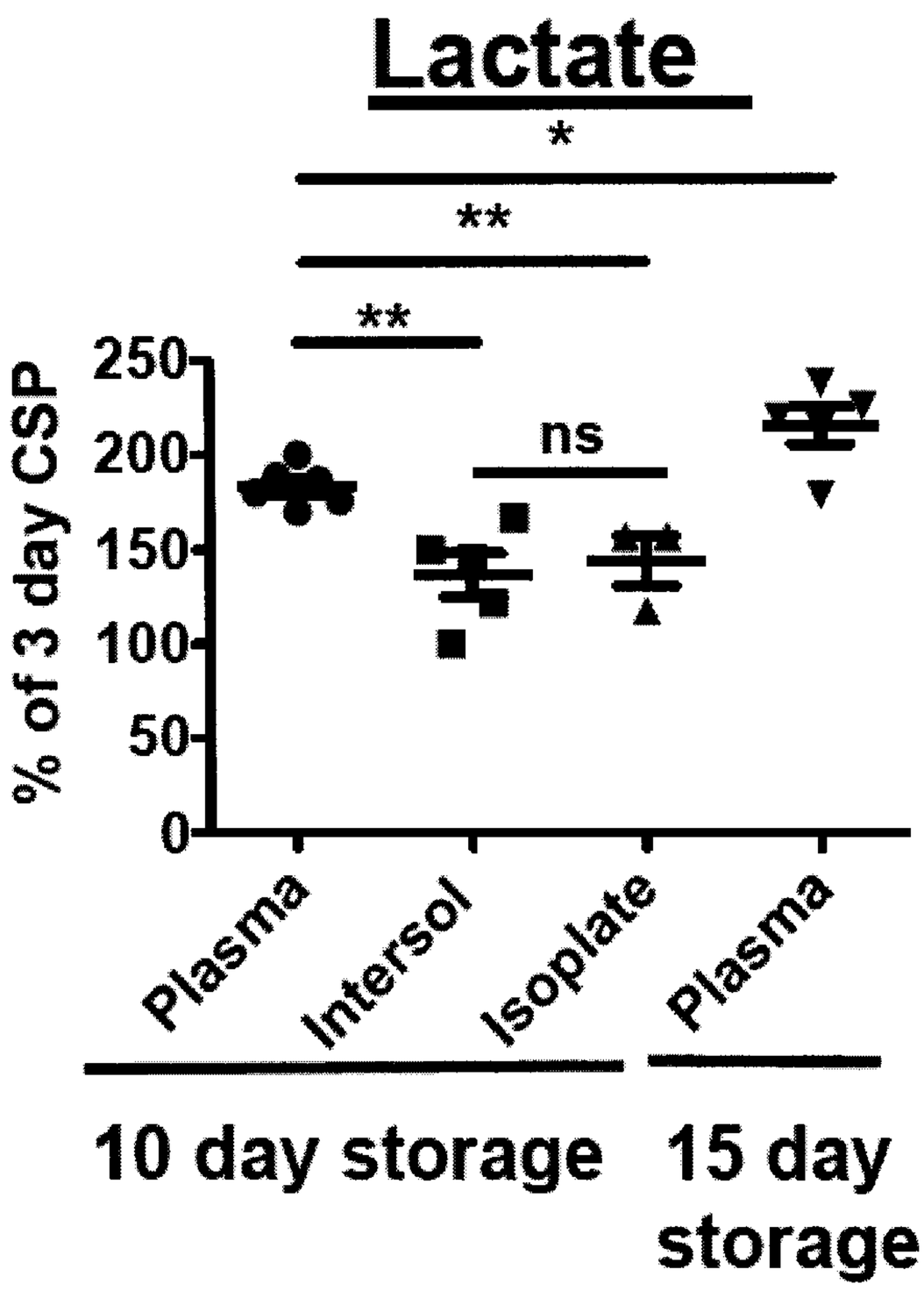

Lactate
*
**
**
ns
% of 3 day CSP
250
200
150
100
50
0
Plasma
Intersol
Isoplate
Plasma
10 day storage
15 day storage

FIG. 2C

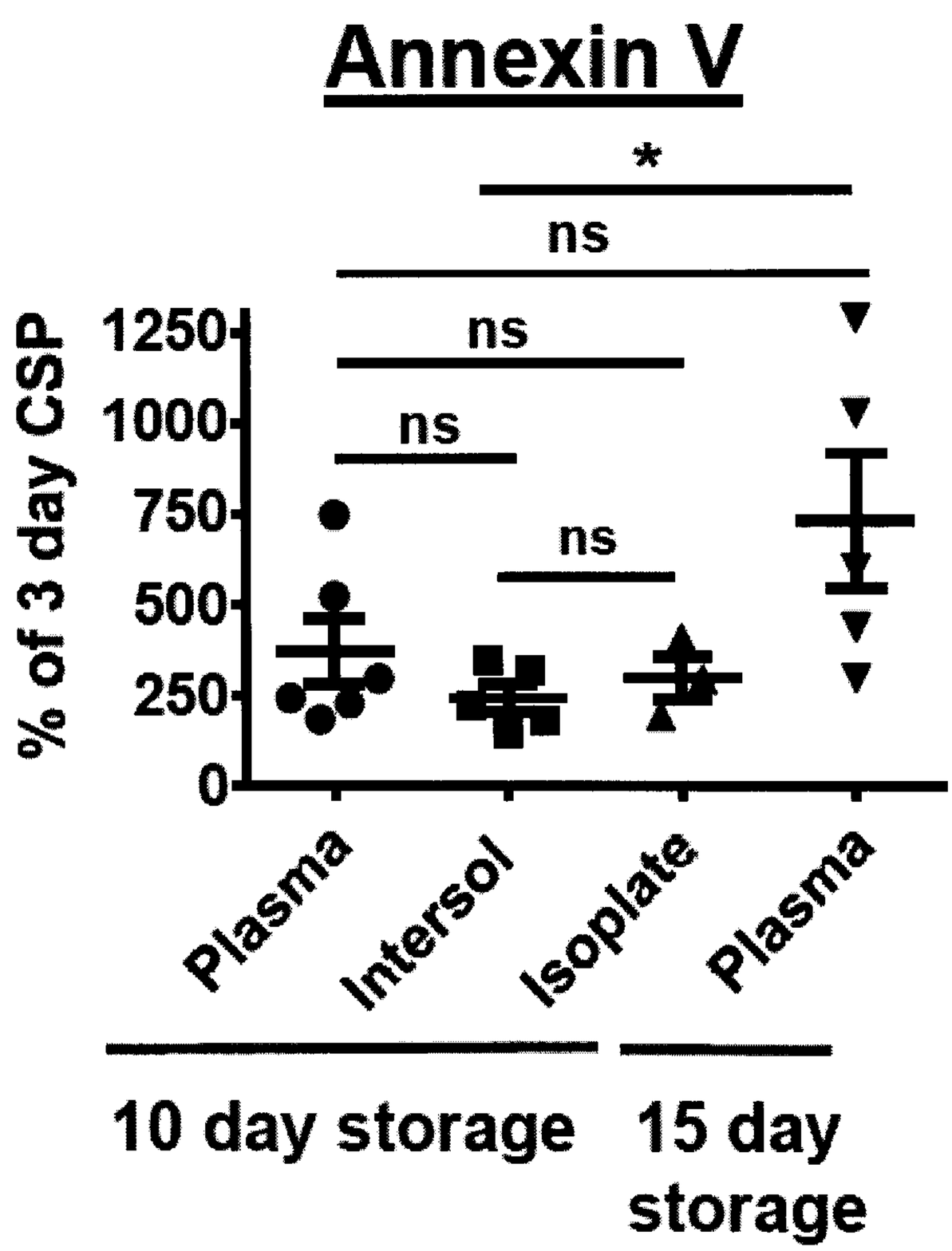

Annexin V
*
ns
ns
ns
ns
% of 3 day CSP
1250
1000
750
500
250
0
Plasma
Intersol
Isoplate
Plasma
10 day storage
15 day storage

FIG. 2D

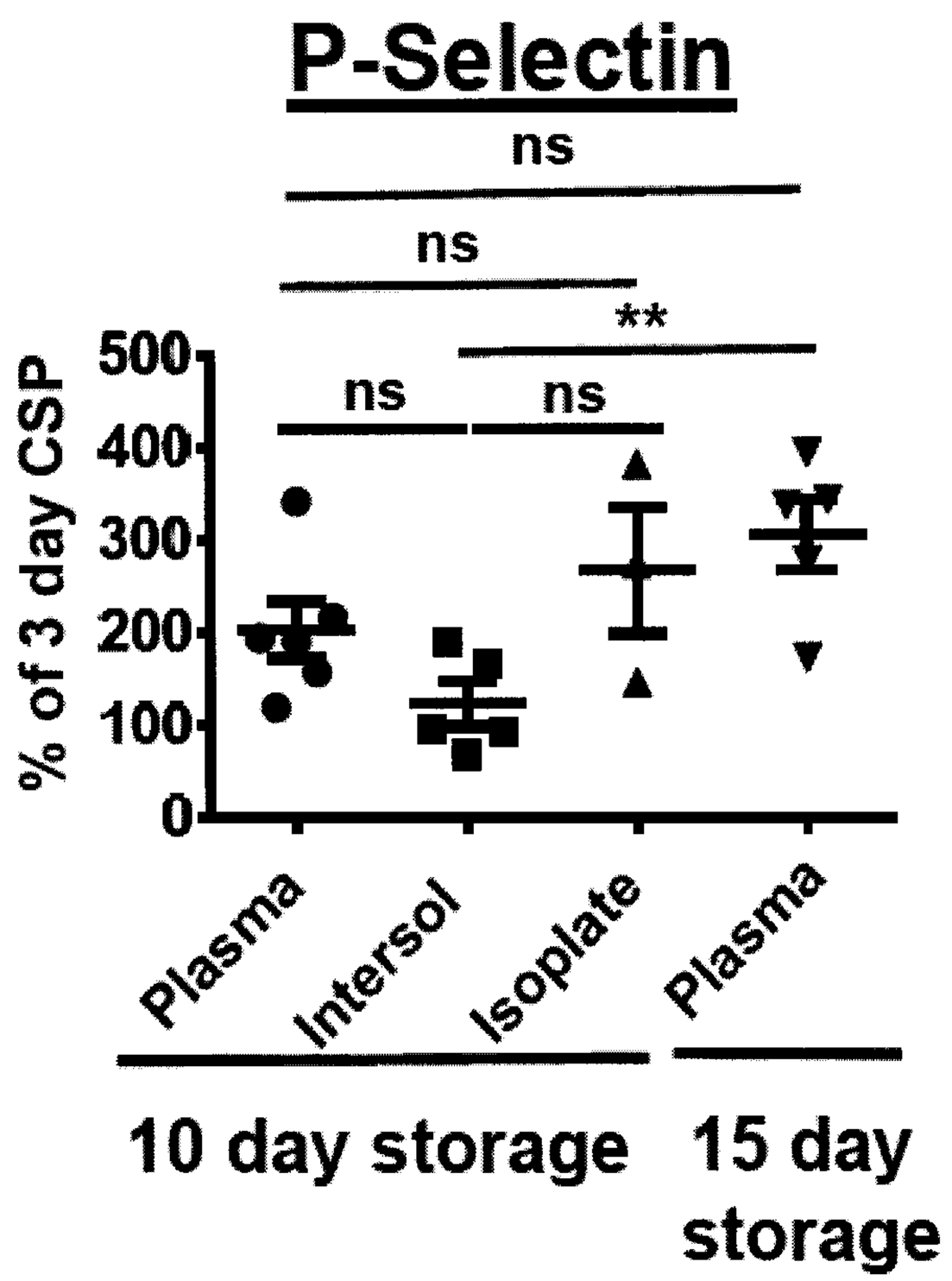

P-Selectin
ns
ns
**
ns
ns
% of 3 day CSP
500
400
300
200
100
0
Plasma
Intersol
Isoplate
Plasma
10 day storage
15 day storage

FIG. 2E

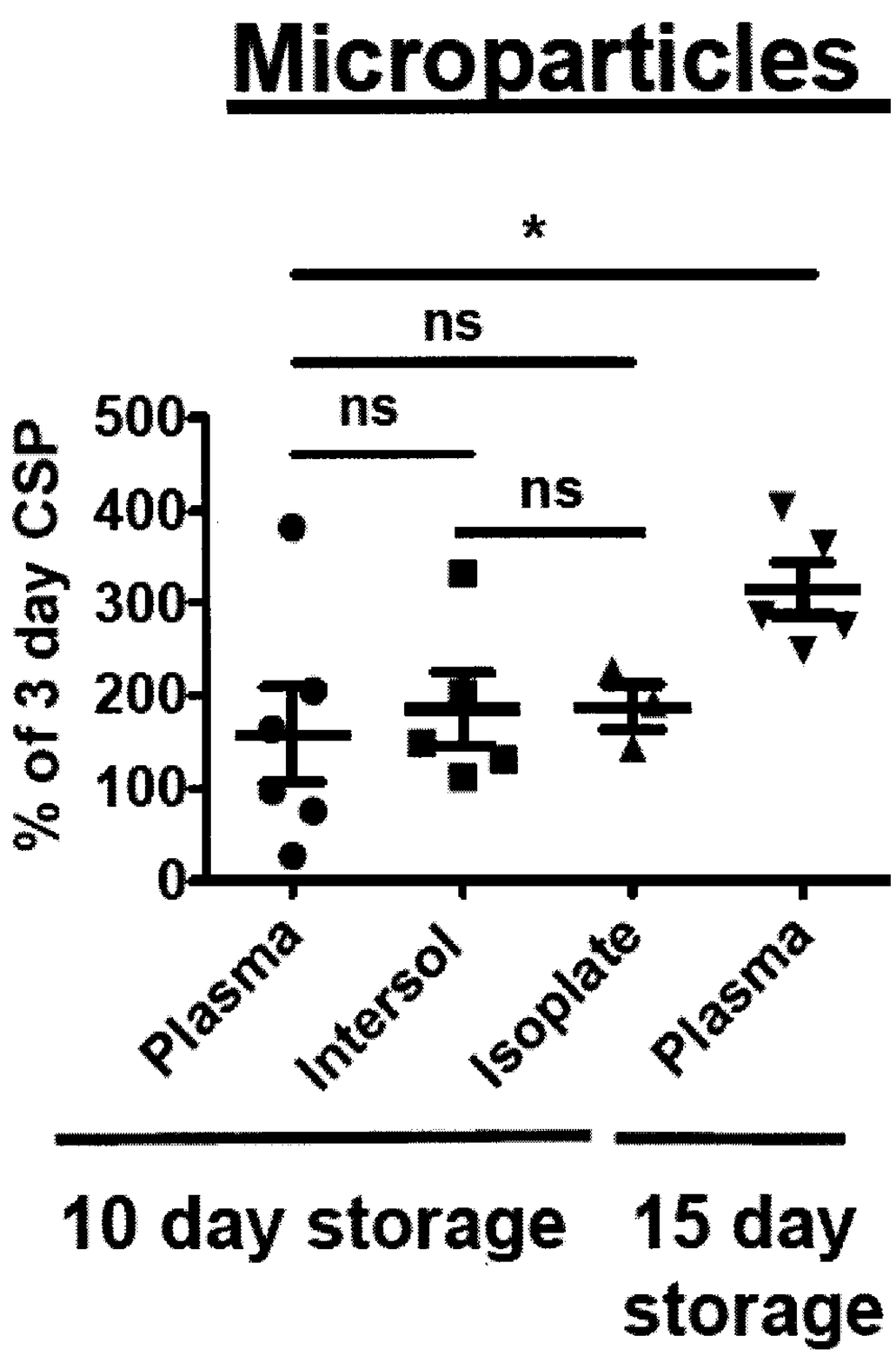

Microparticles
*
ns
ns
ns
% of 3 day CSP
500
400
300
200
100
0
Plasma
Intersol
Isoplate
Plasma
10 day storage
15 day storage

FIG. 3A

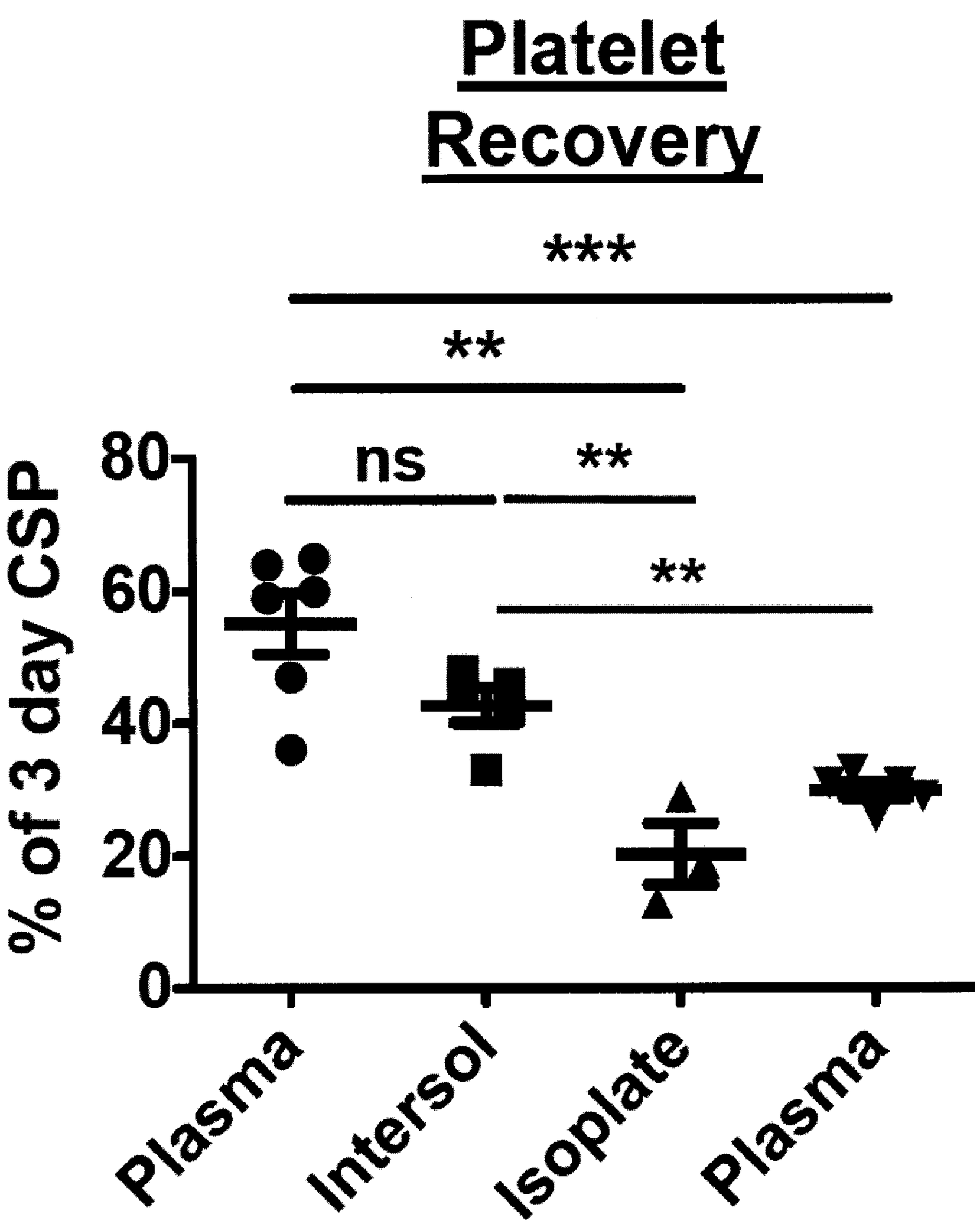

Platelet Recovery
***
**
ns
**
**
% of 3 day CSP
80
60
40
20
0
Plasma
Intersol
Isoplate
Plasma

FIG. 3B

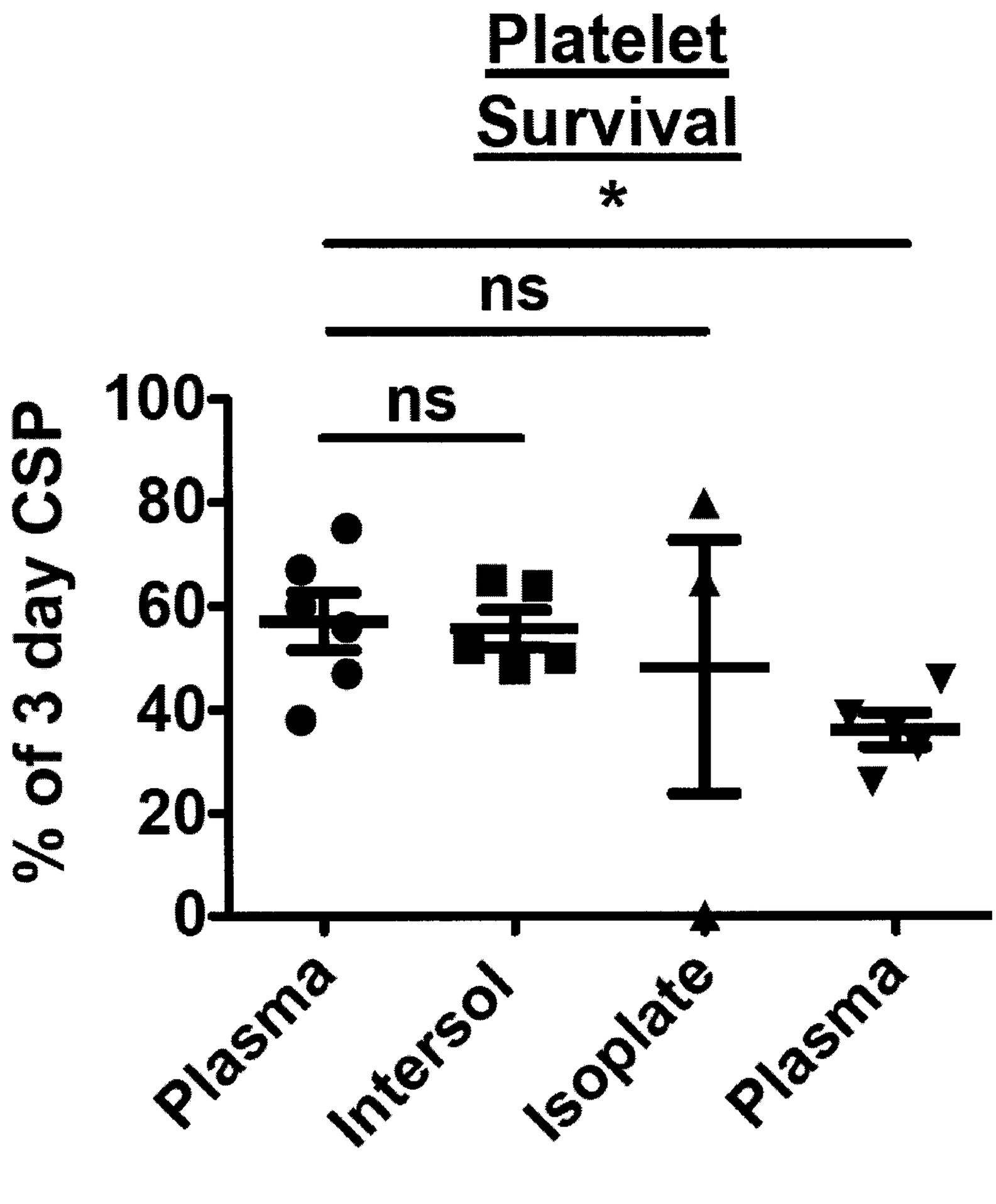
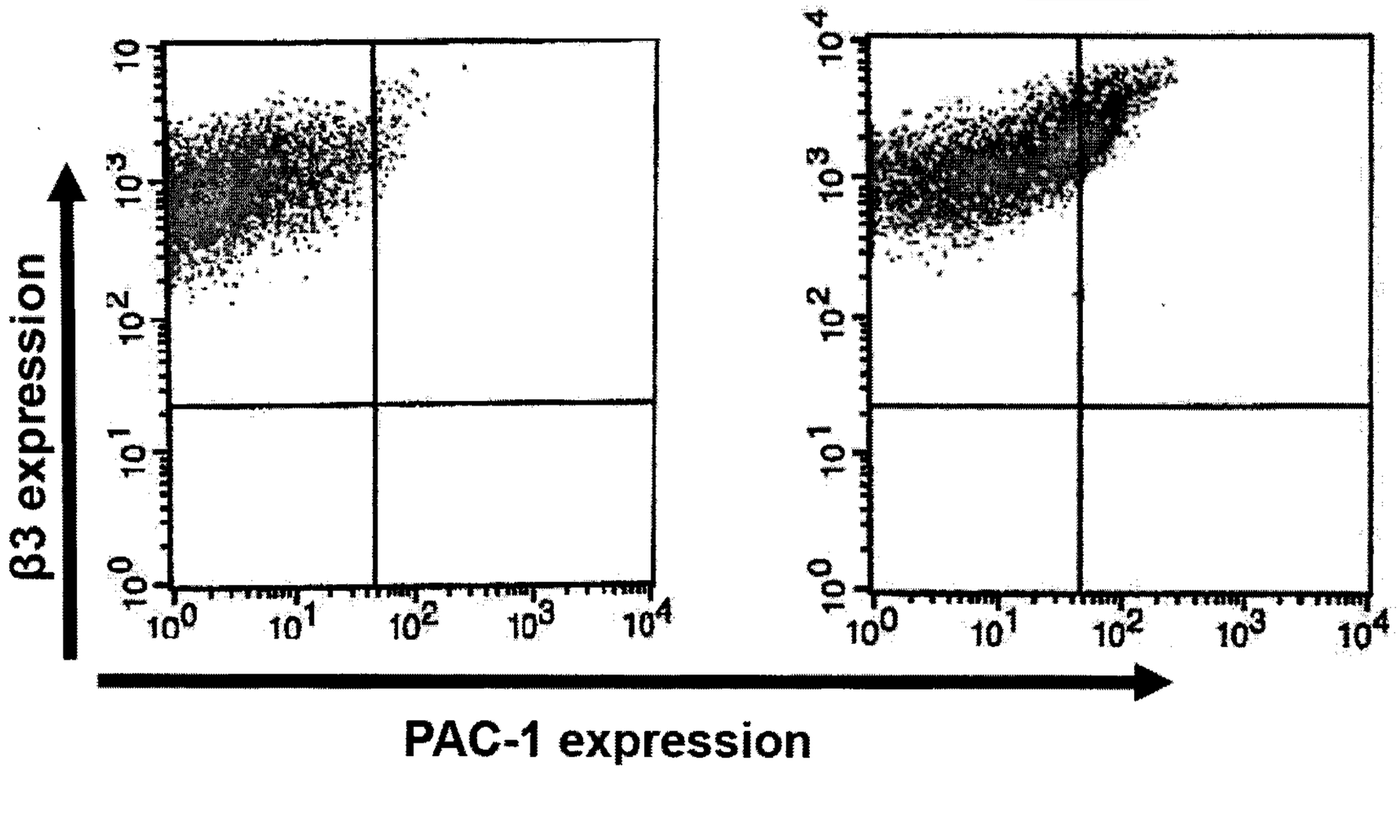

Platelet
Survival
*
ns
ns
% of 3 day CSP
100
80
60
40
20
0
Plasma
Intersol
Isoplate
Plasma

FIG. 4


Baseline
ADP
β3 expression
PAC-1 expression
10^0
10^1
10^2
10^3
10^4