Canine Platelet Concentrates: An *In Vitro* Study to Effectively Provide a Source of Functional Platelets

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CANINE PLATELET CONCENTRATES: AN *IN VITRO* STUDY TO EFFECTIVELY PROVIDE A SOURCE OF FUNCTIONAL PLATELETS

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(ABSTRACT)

This study monitored the storage lesion of 15 units of canine platelet concentrates harvested by differential centrifugation. Canine platelet concentrates were stored at 20-24° C in a platelet rotator for a total of 9 days; the storage lesion of three second generation platelet storage containers was compared. The battery of *in vitro* tests used to monitor the storage lesion were selected from previous studies performed with human platelet concentrates separated by differential centrifugation. Based on these tests, canine platelet concentrates exhibited a storage lesion similar to human platelet concentrates. Metabolic analytes demonstrated decreasing pH, carbon dioxide, bicarbonate and glucose concentrations concurrent with increasing oxygen and lactate dehydrogenase activity over the 9-day period. Platelet structural changes were monitored by mean platelet volume, which began to increase on Day-5. Platelet function appeared to be compromised, as indicated by aggregation studies using collagen and adenosine diphosphate as agonists. Product sterility was maintained.

There was no consensus of data supporting superior performance of one platelet storage container. This study indicates that canine platelet concentrates may be harvested by differential centrifugation of whole blood. *In vitro* studies utilizing three second-generation platelet storage bags support a previous study and concurs that canine platelet concentrates stored at 20-24° C using continuous agitation are viable for at least 5 days.

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Introduction

Interest in transfusion medicine by veterinary professionals was first recorded at the 87th Annual Meeting of the AVMA in 1950.[1] Since this time, advances in human transfusion medicine have led to advances in veterinary transfusion medicine. One benefit of this technology has been in blood component therapy. Separation of a unit of whole blood into components was made possible by the development of plastic bags for blood collection and high speed refrigerated centrifuges.[2] Manipulation of whole blood into blood components is relatively common practice in veterinary medicine and it is not uncommon to find emergency clinics and small animal practices that collect, process and store canine red cells and plasma.[3,4] Guidelines exist for veterinary blood banking techniques in regard to collection and processing of canine fresh whole blood to red cells, fresh frozen plasma, cryoprecipitate and cryo-poor plasma.[1,3] However, techniques for producing platelet concentrates for routine use in veterinary medicine are limited,[5,6] as success in harvesting canine platelet concentrates has only been reported in the last 10 years.[5,6] Currently, fresh whole blood may be used to treat thrombocytopenia in dogs.[6,7] However, platelet concentrates are a superior blood product since they provide a high concentration of platelets while reducing the risk associated with increasing red cell mass, hyperproteinemia and volume overload that may occur due to the infusion of fresh whole blood.[6] Canine fresh whole blood maintains *in vitro* platelet viability for only 6 hours[8] while platelet concentrates have been shown to maintain *in vitro* viability for 5 days.[6]

Differential centrifugation of whole blood to produce platelet concentrates has been used in human medicine for more than 40 years.[9] Current practice in human medicine; however, utilizes apheresis to harvest platelet concentrates from donors. Apheresis equipment is both labor intensive and expensive; therefore, apheresis has not been used in veterinary medicine. Harvesting platelet concentrates from fresh whole blood is the most economical method of obtaining this product; many veterinary practices and veterinary

teaching hospitals already have the equipment needed to separate this component from fresh whole blood.

Metabolic activity of the cellular components of blood continues throughout blood collection, processing and storage. Within platelet concentrates platelet metabolism continues and it becomes the task of the storage environment to support platelet life. During the storage period, a portion of platelets will reach the end of their 5 – 7 day lifespan[10]; however, platelet demise may be exacerbated by the evolving biochemical environment during storage.

Collectively, the biochemical, structural and functional changes that occur during platelet storage under blood bank conditions are known as the storage lesion.[11-13] Storage induced lesions have an impact on platelet viability and hemostatic function.[14] The severity of the storage lesion may be monitored by laboratory tests selected to demonstrate the cumulative effect of these conditions. However, recent improvement of the quality of blood storage bags may diminish these cumulative effects and provide canine platelet concentrates with an extended shelf life.

This study will test the hypothesis that canine platelet concentrates will maintain viability *in vitro* for nine days. The objectives of this study are to establish centrifuge calibration for harvesting platelet concentrates from fresh whole blood, to establish a procedure for harvesting this blood component from fresh whole blood and to monitor *in vitro* viability of platelet concentrates. Three types of platelet storage containers will be compared and evaluated.

Platelet Production, Structure, and Function

Megakaryocytopoiesis

Pluripotential stem cells represent the most undifferentiated of all bone marrow derived cells. These early precursor cells are capable of self renewal or differentiation into one cell lineage.[15,16] Regulation and differentiation of pluripotential stem cells arises from interaction with hematopoietic growth factors which are membrane bound acidic glycoproteins known as interleukins.[15]

Pluripotential stem cells that commit to self renewal remain in the bone marrow. Those stem cells under the influence of stem cell factor and fetal liver tyrosine kinase-3 (*flt*-3) ligand commit to the hematopoietic stem cell line. Upon this commitment, hematopoietic stem cells (also known as myeloid stem cells) have diminished self renewal capacity and restricted lineage potential.[15,16] These cells are known as colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM). These cells are precursors of granulocytes, monocytes, basophils, eosinophils, erythrocytes and megakaryocytes. These hematopoietic stem cells are often identified immunologically by the CD34 antigen, which is a glycoprotein lineage marker of hematopoietic precursors.[15]

Under the influence of granulocyte, monocyte colony stimulating factor (GM-CSF)and hematopoietic growth factors interleukin-3, stem cell factor, and *flt*-3 ligand, CFU-GEMM develops into colony forming unit-granulocyte-macrophage progenitor cells (CFU-GM). Further differentiation of these cells results in development of colony forming unit-granulocyte (CFU-G) and colony forming unit-macrophage (CFU-M) which are precursors of granulocytes and monocytes, respectively.[15] The hematopoietic growth factors interleukin-3, interleukin-6, interleukin-11, stem cell factor, *flt*-3 ligand and thrombopoietin stimulate CFU-GEMM differentiation[17] into colony forming unit-basophil (CFU-B), colony forming unit-eosinophil (CFU-E), blast forming unit-

erythrocyte (BFU-E), and colony forming unit- megakaryocyte (CFU-Meg). These cells develop into basophils, eosinophils, erythrocytes and megakaryocytes, respectively.[15]

Megakaryocyte production is regulated by at least two humoral factors: megakaryocyte-colony stimulating factor (Meg-CSF) and thrombopoietin. Meg-CSF induces the CFU-Meg to proliferate; thrombopoietin promotes differentiation and maturation. The earliest morphologically recognizable form of the megakaryocytic line is the megakaryoblast, maturing in sequence to the promegakaryocyte, granular megakaryocyte and mature megakaryocyte. Megakaryocytic maturation is described morphologically in stages.[15,17] Stage I is the megakaryoblast; it is cytologically described as having a compact nucleus and basophilic cytoplasm when stained with Wright's Stain. Stage II is the promegakaryocyte; it has a horseshoe shaped nucleus and basophilic cytoplasm with red-pink granules becoming visible. Stage III is the granular megakaryocyte, it possesses a multilobed nucleus and its cytoplasm is characterized by diffuse red-pink granules. Stage IV is the mature megakaryocyte; it has a compact yet lobulated nucleus. Its cytoplasm is no longer basophilic and granules are clustered in small aggregates. These small aggregates are defined by invaginated surface membranes that ultimately separate the cytoplasm into individual platelets. Platelets are formed and released from megakaryocytes by a process of cytoplasmic fragmentation; the demarcation lines are formed by infolding of the cytoplasmic membrane. In the bone marrow, mature megakaryocytes are adjacent to sinus walls and platelets are released into the peripheral blood through the bone lumen.[15]

Platelet Structure

The resting platelet is discoid or lentiform in shape, anuclear and has no filopodia.[18] The surface of the platelet is smooth and slightly biconvex in contour.[10] The size of the platelet ranges from 5 to 7 μm long and 3 μm wide.[18] The structure of the platelet may be described by functional zones: the peripheral zone, sol-gel zone and organelle zone.[14] The peripheral zone is the outer region of the platelet, the plasma membrane. The exterior of the plasma membrane is covered by a carbohydrate coat named the

glycocalyx.[18] The glycocalyx is rich in glycoproteins which serve as receptors for various platelet agonists and mediate platelet interactions with other cells and other proteins.[19] GPIIa-IIIb receptors are located here. Glycoproteins are important for platelet adherence, activation, aggregation and subsequent release reaction.[20] The middle layer of the peripheral zone consists of a bilayer of asymmetrically arranged phospholipids that provide a negatively charged surface necessary for interaction between activated platelets and coagulation factors.[14,19] This lipid bilayer contains arachidonic acid, necessary for the synthesis of eicosanoids; eicosanoids are released from activated platelets and can serve to facilitate platelet activation and aggregation.[19] Transmembrane glycoproteins are embedded in this lipid bilayer and serve as receptors for agonist and surface mediated stimuli that trigger platelet activation.[14] Within the platelet plasma membrane, there are numerous infoldings that form the walls of the surface-connected open canicular system (OCS).[14] The OCS is a tubular system that weaves from the peripheral zone throughout the platelet cytoplasm and is in direct communication with the extracellular environment. The OCS is believed to provide a route for excretion of granular contents during activation and exposure of platelet surface receptors during platelet aggregation.[21]

The platelet cytoplasm is located within the sol-gel zone. The cytoskeleton of the sol-gel zone serves as a scaffold that determines cell shape and general organization of the cytoplasm. The cytoskeleton is a dynamic structure that is responsible for all cell movement, including the internal transport of organelles through the cytoplasm. The cytoskeleton is composed of three types of protein filaments: actin filaments, intermediate filaments and microtubules. Actin filaments are located beneath the plasma membrane and provide mechanical support and movement of the cell surface in conjunction with the protein myosin. Intermediate filaments provide mechanical strength to the cell but are not directly involved in cellular movement. Microtubules provide structural maintenance to the cell; they determine cell shape and assist in the intracellular transport of organelles.[22] Upon platelet activation, microtubules are responsible for the

extreme structural changes and intracellular reorganization of the platelet[19] and are composed exclusively of the protein tubulin.[20,22]

Formed elements of the sol-gel zone are classified as the organelle zone and consist of mitochondria, lysosomes and alpha and dense granules. Mitochondria play a critical role in the generation of metabolic energy. Platelets can utilize glucose from circulation via membrane transporters or metabolize intracellular glycogen granules, which are derived from the parent megakaryocyte. Adenosine triphosphate (ATP) is produced via glycolysis; glucose is converted to pyruvate in the cytosol and transported to mitochondria where complete oxidation to CO_2 occurs.[21] However, platelets contain few mitochondria and in their absence, pyruvic acid is reduced to lactic acid and diffused out of the cell.[21] Lysosomes are membrane enclosed organelles that contain acid hydrolases. Lysosomes serve as the digestive system of the platelet[22], which can degrade endocytic material or fuse with the membranes of the OCS and release their contents extracellularly during the process of platelet activation.[18] The α-granules contain a number of different proteins, including coagulation factors: albumin, factor V, fibrinogen; platelet specific proteins: β-thromboglobulin, platelet factor 4; growth factors: platelet derived growth factor, β-transforming growth factor, epidermal growth factor, endothelial growth factor and glycoproteins; α-granule specific proteins: GPIb, GPIIb-IIIa and P-selectin.[18] Dense granules are the storage site for ATP, adenosine diphosphate (ADP), guanosine triphosphate, guanosine diphosphate, calcium and magnesium. Serotonin and its precursor are also stored in the dense granules.[18]

The membrane complexes of the open canalicular system reside within the sol-gel zone. Another membrane complex, the dense tubular system (DTS) is also present in this submembranous zone. Unlike the OCS, the DTS is not open to the platelet surface. The DTS is derived from the endoplasmic reticulum of the parent megakaryocyte. It is a site for arachidonic acid metabolism within the platelet. The dense tubular system serves as a calcium sequestering pump and provides low levels of cytoplasmic calcium in the resting

platelet[18], which in turn maintains the discoid shape of the unstimulated platelet by sustaining polymerization of the microtubules located in the sol-gel zone.[19] Enzymes associated with prostaglandin[14] and eicosanoid synthesis[19] are located within the DTS.

Platelet Function

The platelet is a multifunctional cellular element of the blood system; it sustains vascular integrity, modulates inflammatory response and promotes wound healing after tissue damage. In addition, the platelet is vital for thrombogenesis.[10] However, the most important function of the platelet is its role in hemostasis.[14,21,23] Platelets participate in cessation of hemorrhage, as they are the first cellular element of peripheral blood to react when blood vessels are damaged.[21] Platelets possess potent procoagulant mechanisms that lead to formation of a hemostatic plug at the site of vascular disruption.[23] These mechanisms involve platelet adhesion, platelet activation, platelet aggregation, and granular secretion. These processes may occur simultaneously or independently of each other, but ultimately lead to termination of bleeding.

Platelet Adhesion

Platelet adhesion is the process during which platelets stick to a non-platelet surface.[10,23] Platelet adhesion is initiated by platelet stimulation with one or more agonists. *In vivo*, platelets do not adhere to each other or to the capillary epithelium under normal conditions.[10] Trauma to the vasculature results in vasoconstriction and in the event that vasoconstriction alone does not prevent hemorrhage, platelets respond in one of two ways. Mild trauma to the vascular endothelium results in damaged endothelial cells releasing two agonists: von Wilebrand factor and fibrinogen. Extensive trauma to the vascular endothelium additionally exposes collagen fibers that are agonistic to platelets.[19,21] Within seconds of exposure to agonists, platelet cellular responses are initiated[19] and platelets adhere as a single layer to the site of injury.[21] Platelet adherence to the subendothelium is mediated by platelet glycoprotein receptors, plasma and subendothelial proteins.[21]

During the adhesion process, the responding platelet changes shape; the normally discoid platelet becomes spherical.[10,19] This is achieved through release of intraplatelet calcium, which changes the organization of microtubules.[10] During platelet adhesion and conformation changes, platelets form filopodia.[21] The process of platelet shape change is known as platelet activation.[10,19,21]

Platelet Membrane Activation

The phospholipase C-phosphatidylinositol pathway is involved in platelet adhesion and activation. The phospholipase C-phosphatidylinositol pathway produces diacylglycerol and inositol triphosphate from phosohatidyl inositol biphosphate. Inositol triphosphate causes release of calcium from storage sites which leads to activation of calmodulin-dependent protein kinases resulting in myosin light chain phosphorylation. Thus, this pathway is responsible for platelet conformational change. Stimulation of this pathway is mediated by transmembrane guanosine triphosphate binding proteins or G proteins.[23] Platelet conformational changes expose membrane binding sites for platelet receptors.[23]

Platelet Aggregation

Platelet to platelet adhesion is described as aggregation.[10,23] Aggregation succeeds platelet adherence to the site of injury, and is responsible for the recruitment of additional platelets to the site of vascular disruption, along with their activation and incorporation into the developing platelet plug.[21]

The major protein involved in aggregation is fibrinogen[23]; the intracellular transmitter of aggregation is calcium.[10,21] When resting, GPIIb/IIIa receptors cannot interact with or bind to fibrinogen. However, once platelets are activated, fibrinogen can bind to and crosslink responding platelets.[23] Platelet aggregation is the platelet's basic response to the release of ADP in the presence of calcium. The ADP initially comes from the injured platelet wall, but is later present through the release reaction. Red blood cells also contain and release ADP, but the largest amount is released from the platelet.[10,23]

Release Reaction

The cellular response to ADP is extraversion of the OCS. During this process, known as the release reaction, platelet dense granules and alpha granules fuse with the membranes of the OCS. Dilation and vagination of the OCS extrudes granular contents to the exterior of the cell. Internal contraction of the microtubules and microfilaments accelerate this process by inducing central mobilization of platelet organelles.[10] This causes a profound increase in platelet metabolism and oxygen utilization.[10] The release of material from the dense granules is called release reaction I; the release of α granules and lysosomes is release reaction II.[10,24]

As platelets participate in adhesion, activation and aggregation, the release reaction proceeds concurrently. The agonists released through the release reaction facilitate the cascade of events in which more platelets are attracted to the site of injury, become activated and aggregate to the already forming platelet plug.

Thrombin Formation

Thrombin is a platelet agonist. Both the intrinsic and extrinsic pathways of the coagulation cascade culminate in thrombin formation. Thrombin causes the conversion of soluble fibrinogen to insoluble fibrin polymers. Circulating plasma provides the majority of fibrinogen, as fibrinogen content of platelet alpha granules is low in comparison. However, fibrinogen supplied by the alpha granules can provide additional substrate molecules for thrombin. The action of thrombin in activation of platelets and on fibrin formation result in significant enhancement in the rate and extent of development of the fibrinogen or fibrin bridges between activated platelets.[21] Once fibrin formation proceeds, platelets contract and form a stable hemostatic plug.

Canine Whole Blood Collection

Canine whole blood should be collected using routine phlebotomy protocol.[5,6] For units intended for processing into platelet concentrates, additional considerations should be observed. Owners should be surveyed to ensure that potential platelet donors have not been medicated with any substance that may inhibit platelet function. The 450 mL unit of whole blood should be drawn in no more than 15 minutes time.[11] The phlebotomy should be uneventful and the unit of blood should be handled gently in order to prevent initiation of activation of platelets during collection and processing.[11,14] Units of whole blood to be processed into platelet concentrates should not be cooled towards 1 – 6° C after phlebotomy. Instead, the units should be kept at room temperature in order to preserve platelet function.[5,6,11]

The Storage Lesion of Platelet Concentrates

Metabolic Analytes

Intracellular ATP is needed to maintain platelet integrity. In the unstimulated platelet, ATP is used to maintain osmotic equilibrium and intramembrane phospholipid conformations. During platelet stimulation, ATP is used to support activation and aggregation of the platelet. Glycogen and glucose are the major energy sources for ATP production in the platelet. Platelet glycogen granules are derived from megakaryocytes; platelets can absorb glucose from circulation through membrane glucose transporters.[21] In the platelet, both glycogen and glucose are metabolized to ATP by glycolysis.[10,21,22] In cells that contain abundant mitochondria, oxidative phosphorylation preceeds glycolysis and pyruvic acid is converted to acetyl-coenzyme A. This process occurs within the platelet to a limited extent as platelets contain few mitochondria. However, platelets contain adequate levels of lactate dehydrogenase (LDH) and alternatively, pyruvic acid is reduced to lactic acid and is diffused out of the platelet.[21]

Metabolism of glycogen and glucose continues during storage of platelet concentrates. Intracellular glycogen and the residual plasma within the platelet concentrate which

contains glucose are sufficient for 5 day storage of human platelets.[25] Platelet metabolism during the storage period eventually depletes these stores.[6,12] *In vivo*, lactic acid is diffused out of the platelet into the circulatory system where it is buffered by bicarbonate and converted to sodium lactate and carbonic acid. *In vitro*, this process is limited to the buffering capacity of the storage system. Once bicarbonate is exhausted, lactic acid accumulates. There is an inverse relationship between pH and lactic acid concentration; as lactic acid increases, pH decreases. This has been reported to be lethal for stored platelets if the pH falls below 6.0.[27]

In-vitro, platelet death leads to a lower platelet count within the platelet concentrate. Platelet disintegration results in lysis of the cell and discharge of cytosolic compounds including LDH. LDH is used as an in vitro marker for platelet demise[28,29] as significant correlations have been shown when comparing the degree of LDH released and platelet survival within stored platelet concentrates.[30]

Physical Factors Affecting Metabolic Analytes

Storage Devices

Standards issued by the American Association of Blood Banks (AABB) and College of American Pathologists (CAP) specify that human platelet concentrates must maintain an acceptable pH throughout storage. Acceptable pH is defined as not less than 6.2[11,31], and this is directly dependent on the type of plastic bag in which the platelets are collected and stored. Plastic blood collection bags used for storage of platelet concentrates are composed of polyvinyl chloride (PVC) and plasticizers. Plasticizers are used to make PVC more malleable, flexible, and to alter the gas exchange properties of PVC. PVC is a vapor barrier and the addition of plasticizers make the plastic bags more permeable to oxygen and carbon dioxide. First generation platelet storage bags were initially available in the mid-nineteen seventies. These bags contain di(2-ethylhexyl)phthalate (DEHP) as a plasticizer. In the early seventies, it was reported that significant quantities of phthalates could migrate from PVC blood bags into stored blood and localize in human tissue, even

though the ability of humans to metabolize phthalates was unclear at the time. This finding lead to the production of second generation platelet storage bags. Second generation platelet storage bags were introduced in the early nineteen eighties; these bags contain less or no DEHP than first generation containers. These containers also have enhanced oxygen and carbon dioxide diffusion, which assists in preventing a decrease of pH.[27]

Two manufacturers of blood collection systems in the United States provide blood collection systems with platelet storage bags: Baxter Healthcare Corporation, Fenwal Division, and Terumo Medical Corporation. In the Baxter/Fenwal product line, PL 146® is the plastic used for red cell storage. Although PL 146® is now used for red cell storage, it is a first generation platelet storage bag. Two second generation platelet storage bags are available from Baxter/Fenwal: PL 1240 is a multipurpose plastic containing PVC and the plasticizer trimellitate; it is twice as permeable to oxygen and carbon dioxide than PL 146®. PL 732® is composed of polyolefin plastic available from Baxter/Fenwal; it is four times as permeable to oxygen and carbon dioxide than PL 146®. Terumo's platelet container XT612® is composed of PVC and uses DEHP as the plasticizer. However, this container is modified from the first generation of platelet storage bags through the reduction of the thickness of the plastic from the first generation platelet bags. This modification allows for enhanced permeability of oxygen and carbon dioxide through the container, approximately two times that of Fenwal's PL 146®.[27] Each of these containers are currently FDA approved for human platelet concentrate storage for 5 days.[11,25]

Plasma volume

In 1976, Slichter and Harker demonstrated that pH of human platelet concentrates could be maintained above 6.0 if the platelet count of the concentrate was below 1.7×10^{12}/l. Platelet concentrates diluted in 50 mL of plasma showed no change in pH when measured after storage for 48 hours. Seventy milliliters was suggested as the optimal amount of residual plasma for platelet concentrate.[32] AABB currently states that sufficient plasma

remain with the platelet concentrates to maintain the pH of 6.2 or higher for the entire storage period. For storage temperatures of 20-24° C, this usually requires a minimum of 35 mL, but 40-60 mL is recommended.[11]

Storage Temperature and Agitation

Platelet concentrates should be stored at controlled room temperature with continual, gentle agitation.[11,31,32] This is accomplished by the use of a platelet incubator with a built in rotor for storage of platelet concentrates. In 1969, it was demonstrated that cold temperatures markedly reduced post transfusion viability[32,33], as guidelines prior to 1969 called for storage of platelets at the same temperature as red cells. During this time it was also demonstrated that platelet concentrates stored at room temperature were hemostatically more effective in vivo than those stored at 1 – 6 ° C.[26] Continuous mixing of platelet concentrates was found to have a critical effect on the viability of platelets stored at 22 ° C for 24 hours.[32] Agitation facilitates the influx of oxygen and the efflux of carbon dioxide across platelet storage containers and hence, assists in maintenance of pH.[25,34,35] It is the current AABB recommendation that platelets be stored at 22 – 24 ° C with continuous agitation.[11]

Platelet Yield

The AABB recommends that platelet concentrates prepared from a single unit of whole blood contain at least 5.5 X 10^{10} platelets in 75% of all units tested. Platelet yield is dependant on the speed and duration of centrifugation of the unit of whole blood. Whole blood used to harvest platelet concentrates should be centrifuged at the lowest speed and shortest time that results in the highest platelet yield and the lowest red cell yield. This protocol is not standard for all centrifuges, and each centrifuge used to harvest platelet concentrates should be calibrated for optimum platelet harvest.[36]

Platelet rich plasma is separated from whole blood using a light spin. AABB defines light spin as 2000 x g for 3 minutes, although ranges of 1000 – 2600 x g for 3 – 9 minutes

have been reported as producing satisfactory yield.[25] Platelet concentrates are separated from platelet poor plasma using a hard spin, defined as 5000 x g for 5 minutes.[36]

Platelet Structural Changes

A change in morphology occurs during storage of platelet concentrates. This is due to the fact that platelets lose their ability to maintain osmotic equilibrium, resulting in platelet swelling accompanied by shape change. These changes may be monitored by variation in the platelet index, mean platelet volume (MPV).[37] MPV is derived using automated instrumentation that calculates the weighted average volume of a platelet in a specified size channel.[38] An increase in MPV indicates an increase in platelet volume and change in shape from discoid to spherical[39], and suggests deterioration within the platelet concentrate.[30] Platelet activation in platelet concentrates is also associated with an increased MPV.[37] MPV has been demonstrated to be inversely related to pH in platelet concentrates[30,40] also indicative of product demise.

Platelet Functional Changes

Aggregation Studies

In vitro, aggregation studies may be performed photometrically using a platelet aggregometer. The Born type, or Optical Aggregometer, is a fixed wavelength double beam spectrophotometer in which infra-red light is passed through a continuously stirred citrated platelet rich plasma sample (the test sample) and a platelet poor plasma sample (the reference sample). When an agonist is added to the platelet rich plasma sample, platelet aggregation is monitored by changes in light transmittance. The rate and degree of aggregation is plotted using a recording device.[41] Platelet discoid to spherical shape change is detected as an initial decrease in transmittance; platelet aggregation allows an increased amount of light to pass through the suspension and is detected as an increased light transmittance.[20] Percent aggregation is calculated.

ADP, epinephrine, collagen, arachidonic acid, thrombin and ristocetin are platelet agonists commonly used to diagnose platelet disorders.[20,42,43] Selection of the agonist(s) tested in aggregation studies is dependant on which agonist receptor or signaling pathway is to be investigated.

In vivo, ADP and epinephrine are contained within the platelet in storage organelles and are released from the platelet during formation of the primary hemostatic plug and induce subsequent aggregation. Collagen is not contained in the platelet but is found in the supporting connective tissue of the blood vasculature and is considered to be the first aggregating or procoagulant factor that the platelet encounters following trauma.[43] ADP, epinephrine and collagen are used in vitro for diagnosing receptor defects of the platelet.[20] Arachidonic acid is important for structural and functional integrity of the platelet cell membrane and is the precursor for eicosanoids[44], and is used in aggregation studies to diagnose disorders of secretion or signal transduction.[20] Thrombin causes the conversion of soluble fibrinogen to insoluble fibrin polymers that ultimately lead to the cessation of bleeding and is used for the diagnosis of receptor defects.[20] Ristocetin is an antibiotic and causes direct agglutination of platelets related to the interaction between von Willebrand cofactor, GPIb and the antibiotic on the platelet surface.[45] Ristocetin is used in platelet aggregation testing to diagnose von Willebrand's disease.[46]

Hypotonic Shock Response

Platelet response to hypotonic shock also correlates with *in vivo* viability and platelet function. Response to hypotonic shock tests the ability of platelets to respond to an influx of water and to recover to their initial shape.[47] Recovery depends on the integrity of the platelet membrane and its volume regulating capabilities[48] and is a measurement of functionality.[47,48] To perform this test, an Optical Aggregometer is used. When water is added to platelet rich plasma, the platelets swell, resulting in a decrease in refractive index and light absorbance. With normal membrane integrity and energy metabolism, platelets are able to extrude the water and regain their normal volume. This extrusion is accompanied by an increase in refractive index and light absorbance.[48]

Bacterial Contamination

Bacterial contamination of blood products has been reported extensively in human medicine[11,49] and on a limited basis in veterinary medicine.[6,50] It is imperative to maintain sterile platelet concentrates during storage since infusion of bacterially contaminated products may result in death of the recipient. Introduction of bacterial contaminates into platelet concentrates may occur from one or more of the following sources:

1. The Donor – Donors may be asymptomatic or may have a low grade infection following dental or other medical procedures. In addition, blood may contain low levels of bacteria that are not necessarily pathogenic to the donor or recipient.[49]
2. During Whole Blood Collection through the Phlebotomy Site – Approximately 80% of the time a needle is placed into a vein, a piece of skin is aspirated through the needle. Bacteria reside in areas of the skin, such as sweat glands and hair follicles where routine pre-phlebotomy cleansing may not be bactericidal.[49]
3. During Whole Blood Processing –Blood collection systems are classified as "open" or "closed." An open system exposes its contents to air or outside elements at some point during collection, processing or storage, and is more prone to bacterial contamination than closed systems. A closed system does not expose its contents to air or outside elements during collection, processing or storage and use of a closed system decreases the risk of bacterial contamination.[11]
4. During Platelet Storage – If bacteria are present, proliferation may be accelerated by platelet room temperature storage and length of storage time.[49]

Experimental Plan

Blood Donors

Twenty blood donors from the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) Veterinary Teaching Hospital (VTH) Canine Blood Bank were used for this study. These dogs were qualified donors of the VTH blood donor program during the course of this study. Qualification was based on weight, age, physical exam, and laboratory test results which were found to be within normal limits for: complete blood count, chemistry profile, occult heartworm, *Babesia canis*, *Erlichia canis*, *Brucella canis* and fecal floatation. This study was approved by the Virginia Polytechnic Institute and State University Animal Care Committee and the VMRCVM, VTH.

Blood Collection Systems

Three blood collection systems were evaluated for collection and storage of platelet concentrates. These were quadruple pack systems containing 63 mL of Citrate Phosphate Dextrose (CPD) anticoagulant solution in the primary bag. Platelet bags were PL 1240[a], PL 732® [b] or XT612®[c]. PL 1240 consists of polyvinyl chloride (PVC) and the plasticizer trimellitate, PL 732® is composed of polyolefin plastic and XT612® is composed of PVC and utilizes di(2-ethylhexyl)pthalate (DEHP) as a plasticizer. Each of these bags is currently approved for 5-day storage of human platelets.[25]

Blood Collection

One unit (450 mL) of whole blood was collected from each donor by jugular phlebotomy using a vacuum chamber[d] at a pressure less than 5 mm Hg. Total blood collection time was monitored. Once collected, the unit of whole blood was kept at room temperature and processed immediately for separation into components.

Centrifuge Calibration

Five units of whole blood were used to calibrate the VTH Blood Bank centrifuge[f]. Successful preparation of platelet components requires adequate but not excessive centrifugation.[36] Centrifuge calibration is necessary since centrifuge speed (in revolutions per minute) and product centrifugation time are not universal for all centrifuges. Two centrifuge speed settings were needed in order to process platelet concentrates, a light spin and a heavy spin. Light spin is defined as 2000 x g for 3 minutes and is used for obtaining platelet rich plasma (PRP) from whole blood. Heavy spin is defined as 5000 x g for 5 minutes and is used to concentrate PRP to platelet concentrate (PC). In order to calculate revolutions per minute for the centrifuge speed setting, the following formula was used[51]:

rcf (in g) = $28.38R\ (rpm/1000)^2$, where rcf = relative centrifugal force, R = radius of centrifuge rotor in inches (10.9 inches), rpm = revolutions per minute. Thus, light spin (2000 x g) was calculated to 2543 rpm and heavy spin was calculated to 4020 rpm. The following procedure was used for centrifuge calibration.

Light Spin

Three units of whole blood were used to calibrate for light spin. Platelet counts were determined for each unit of whole blood. PRP was prepared from one unit of whole blood using the above calculated light spin for 3-minute duration. PRP was prepared from a second unit of whole blood by increasing the centrifuge speed by 30% (3369 x g). A third unit of whole blood was prepared using a 5000 x g for 3 minutes. The platelet count of each PRP was determined. Percent yield was calculated as follows:

Total Number of Platelets in the unit of whole blood = platelet count of whole blood per μL X 1000 μL per mL X number of mL of whole blood

Total number of platelets in PRP = platelet count of PRP per μL X 1000 μL per mL X number of mL of PRP

PRP percent yield = number of platelets in PRP X 100 / number of platelets in whole blood

The centrifugation setting that resulted in the highest percent platelet yield with acceptable levels of red blood cell content was established as light spin for the VTH Blood Bank centrifuge[f] and was used for processing whole blood to platelet rich plasma for the remainder of the study. The units of whole blood and PRP used for calibration studies were not used for *in vitro* studies.

Heavy Spin

The VTH Blood Bank centrifuge was previously calibrated for the heavy spin, since this setting is used for separation of plasma from whole blood. This setting was used for processing platelet rich plasma to platelet concentrate.

Blood Processing

Prior to centrifugation, one heat sealed[e] segment of CPD anticoagulated whole blood was removed from each unit to obtain the red cell count, platelet count and mean platelet volume (MPV).

Component Separation

The remaining 15 units of whole blood were centrifuged at the established light spin at 20 – 24 ° C. Between 175 - 230 mL of platelet rich plasma was expressed from the red cells into the integrally attached platelet bag using a plasma extractor[g]. The primary bag containing the residual red cells and the group of three satellite bags, one of which contained platelet rich plasma, were separated by heat seal. The red cells were stored as VTH Blood Bank stock. The platelet rich plasma was weighed[h] and satellite bags were centrifuged at the established heavy spin for 5 minutes at 20 – 24 ° C. This spin concentrated the platelets within the platelet rich plasma into a platelet pellet. An average of 144 mL of plasma was left with the platelet pellet; excess platelet poor plasma was expressed from the platelet concentrate bag into one of two integrally attached

satellite bags. The bag containing the platelet pellet was separated from the two satellite bags by heat seal and weighed. The remaining platelet poor plasma was frozen as VTH Blood Bank stock. The bag containing the platelet pellet was left label side down, undisturbed at room temperature for 60 minutes to promote disaggregation of platelets.[5,52] After this time, a sampling coupler device[i] was aseptically spiked into the platelet bag to enable sampling of platelet concentrate for subsequent testing. The platelet concentrates were placed in a platelet incubator/rotator[j] at 20 – 24 ° C for two hours to promote resuspension prior to testing. Platelet concentrates remained in the platelet incubator/rotator for the duration of the study.

Weight measurements were converted to volume measurements using the following conversion factors: 1 mL whole blood or red cells = 1.06 grams (g), 1 mL platelet rich or platelet poor plasma = 1.03 g.

Sample Collection

A total of 15 mL of sample was aseptically removed from each platelet concentrate on Day 0 via the sample site coupler. The coupler was cleansed with an alcohol prep; a 23 gauge needle attached to a 3 mL syringe was inserted. Two mL of platelet concentrate was drawn and discarded. Leaving the needle in place, 11 mL of platelet concentrate was drawn in a 12 mL syringe and an additional 2 mL was drawn in a 3 mL syringe. The needle attached to the 3 mL syringe was sealed upon withdrawal from the sample site coupler in order to minimize specimen exposure to atmospheric air. The sample site coupler was cleansed with an alcohol prep and the platelet concentrate was returned to the platelet incubator/rotator. On Days 3, 5, 7 and 9, the same protocol was used, but sample volume was decreased: 2 mL of platelet concentrate was drawn and discarded, 8 mL of platelet concentrate was drawn in a 12 mL syringe and an additional 2 mL was drawn in a 3 mL syringe.

The samples were analyzed Day 0, 3, 5, 7 and 9 for pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), bicarbonate, platelet count, mean platelet

volume, white blood cell count, red blood cell count, glucose, lactate dehydrogenase activity, microbial culture, platelet hypotonic stress response and platelet aggregation. Additionally on Day 0, red blood cell count and total lactate dehydrogenase lysis were performed; red blood cell contamination rate and platelet yield were calculated.

Measurement of pH, pO_2, pCO_2, bicarbonate, platelet count, mean platelet volume, red blood cell count, and glucose

The sealed 2 mL syringe sample was used for measurement of pH, pO_2, pCO_2 and bicarbonate. These parameters were measured at 37 ° C using a blood gas analyzer[k] and results were temperature corrected to 22 ° C. The remaining sample was analyzed for platelet count, mean platelet volume and white blood cell count using an automated hematology analyzer[l]. Additionally, red blood cell count was obtained on Day 0. The sample was then centrifuged for 5 minutes at 621 x g; glucose concentration was measured from the supernatant using an automated chemistry analyzer.[m]

Percent LDH Activity

LDH Lysis: In order to determine the maximum LDH activity of each platelet concentrate prior to storage, a sample obtained from each platelet concentrate on Day 0 was incubated with a lysing agent[n]. Platelet lysis resulted in discharge of cytosolic compounds including LDH, which is used as an *in vitro* marker for platelet demise. On Day 0, 450 µL of platelet concentrate was incubated at 37 ° C with 5 µL of a lysing agent[n] for one hour. After incubation, the sample was centrifuged for 5 minutes at 621 x g and LDH was analyzed on the supernatant using an automated chemistry analyzer. This provided a baseline of total LDH activity to compare with platelet concentrate LDH activity after storage.

LDH Activity: On Day 0, 3, 5, 7 and 9, 250µL of prostaglandin E_1[p] was added to 1 mL of platelet concentrate and centrifuged for 5 minutes at 621 x g. Prostaglandin E_1 was used to stabilize and inhibit activation of platelets after sampling and during centrifugation. LDH was analyzed from the supernatant. Percent LDH activity was

calculated for Day 0, 3, 5, 7 and 9 for each unit of platelet concentrate using the following formula: (LDH Activity of Day *x* / LDH Lysis) x 100, where *x* = 0, 3, 5, 7 or 9.

Platelet Aggregation

Platelet aggregation studies were performed using a platelet aggregometer[q]. To obtain the platelet poor plasma specimen, one milliliter of platelet concentrate was centrifuged at 621 x g for 5 minutes; 500µL of this specimen was pipetted into an aggregometer microcuvette and incubated in the platelet poor plasma well in the aggregometer for 5 minutes. The platelet rich plasma specimen was obtained as follows. If the platelet count of the original specimen obtained from the platelet concentrate was above 300,000/µL, the platelet count was adjusted to 200,000-300,000/µL using platelet poor plasma as the diluent. This mixture was left undisturbed for 30 minutes prior to testing and used as platelet rich plasma for this procedure; otherwise, the original undiluted specimen was used. Five hundred microliters of platelet rich plasma was placed in each of two aggregometer microcuvettes and incubated for 5 minutes. A stir bar was inserted into the warmed platelet rich plasma samples and allowed to stir for 5 minutes prior to testing. This microcuvette was placed in the appropriate test well of the aggregometer and a baseline reading was obtained. Ten µM (5 µL) of ADP[r] and 2 µg/mL (1 µL) of collagen[s] were consecutively and forcefully added to the platelet rich plasma. Response was measured for a minimum of 4 minutes. The highest plateau of the aggregation curve was recorded. This value was subtracted from the baseline value and reported as percent aggregation.

Platelet Hypotonic Shock Response

The hypotonic shock response (HSR) was performed using a platelet aggregometer. Platelet poor and platelet rich plasma specimens were obtained and incubated as described in the platelet aggregation procedure. A baseline reading was obtained. Two hundred fifty microliters of prewarmed phosphate buffered saline[t] (PBS) was forcefully injected into the platelet rich plasma microcuvette. The reaction was recorded for at least

30 seconds and this platelet rich plasma sample was discarded. The second platelet rich plasma sample was inserted into the appropriate test well and baseline was established. Two hundred fifty microliters of prewarmed sterile water[u] was forcefully injected into the platelet rich plasma microcuvette. The initial reaction with water was immediately observed; the reaction was recorded for 4 minutes. Initial reaction with saline, initial reaction with water and response at 4 minutes were used to calculate percent recovery as follows:

Response at 4 minutes / (Initial reaction with water – initial reaction with PBS).

Microbiological Evaluation

Five hundred microliters of platelet concentrate was added to 5 mL of Brain Heart Infusion broth[v] on Day 0, 3, 5, 7 and 9. This mixture was incubated at 35 ° C for 24 hours, and then plated to Blood Agar[w], MacConkey[x], and Brucella[y] plates. Aerobic and anaerobic culture results were reported at 72 hours.

Analysis of Data

Platelet yield and red blood cell contamination were calculated. Mean analyte values were calculated for each day and bag type. The ANOVA model for repeated measure was used to evaluate changes in analyte values due to the effects of bag type, day and the interaction of bag type and day, using the mixed procedure of the SAS System[z]. When significance was detected, further investigation was performed using the slice option to test the simple effect of day and was controlled using Bonferroni's correction. Percent platelet aggregation and percent LDH lysis were log transformed to stabilize variance; confidence intervals were calculated. For microbiological evaluation, any growth was considered significant; otherwise, the level of significance was set at $p < 0.05$.

Results

Centrifuge Calibration

Centrifuge settings of both 2500 and 3330 rpm produced yields greater than 75% (Table 1). However, the acceptable red blood cell contamination rate based on visual inspection, was achieved using the centrifuge setting of 3330 rpm (Table 2). Therefore, platelet rich plasma light spin was established at 3369 x g for 3 minutes duration for the VTH Blood Bank centrifuge.[f]

Table 1. Light Spin Centrifuge Calibration

Donor Number	CPD plt	mL WB	plts in WB	PRP plt	mL PRP	plts in PRP	% yield	rpm
328	139,000	450	6.26×10^{10}	271,000	202	5.47×10^{10}	87.52	2550
331	202,000	450	9.09×10^{10}	413,000	175	7.23×10^{10}	79.51	3330
286	194,000	450	8.73×10^{10}	273,000	175	4.78×10^{10}	54.73	4020

CPD plt : platelet count of citrate phosphate dextrose whole blood /µL
mL WB : milliliters of whole blood
plts in WB : number of platelets in whole blood; =CPD plt X 1000 µL/mL X mL WB
PRP plt : platelet count of platelet rich plasma/ µL
mL PRP : milliliters of platelet rich plasma
plts in PRP : number of platelets in platelet rich plasma; = PRP plt X 1000 µL/mL X mL PRP
% yield : percent yield = (plts in PRP X 100) / plts in WB
rpm = (square root of (rcf / 28.38R)) X 1000 ; where rpm = revolutions per minute, rcf = relative centrifugal force (in g), R = radius of centrifuge rotor in inches (10.9 inches)

Table 2. Red Blood Cell Contamination

Unit	CPD RBC	mL WB	RBC in WB	RBC PRP	mL PRP	RBC in PRP	Percent contamination	rpm	color
328	5.610E+06	450	$2.525x10^{12}$	290,000	202	$5.858x10^{10}$	2.32	2550	red
331	6.480E+06	450	$2.916x10^{12}$	244,000	175	$4.270x10^{10}$	1.46	3330	straw
286	6.480E+06	450	$2.916x10^{12}$	190,000	175	$3.325x10^{10}$	1.14	4020	straw

CPD RBC : red blood cell count of citrate phosphate dextrose whole blood/ µL
mL WB : milliliters of whole blood
RBC in WB : number of red blood cells in whole blood; = CPD RC X 1000 µL/mL X mL WB
RBC PRP : red blood cell count in platelet rich plasma / µL
mL PRP : milliliters of platelet rich plasma
RBC in PRP : number of red blood cells in platelet rich plasma; = RBC PRP X 1000 µL/mL X mL PRP
Percent contamination = (RBC in PRP X 100) / RBC in WB
rpm = (square root of (rcf / 28.38R)) X 1000 ; where rpm = revolutions per minute, rcf = relative centrifugal force (in g), R = radius of centrifuge rotor in inches (10.9 inches)

Blood Donors and Collection Systems

The population of blood donors used for this study were comprised of mixed and pure breed dogs of varied dog erythrocyte antigen (DEA) blood type (Table 3). Units of blood were collected as necessary to maintain VTH Blood Bank Red Cell stock levels; between 1 and 4 units of blood were collected per week for a period of 5 weeks. Each blood collection system was alternated to avoid consecutive phlebotomies of one bag type. Each blood collection system was used 5 times for a total of 15 phlebotomies.

Table 3. Blood Donor Dog Erythrocyte Antigen (DEA) Type and Breed

Platelet Storage Container	DEA Type			Breed	
	1.1	1.2	Universal	Mixed	Other
PL1240[a]	2	1	2	3	1 Akita 1 Doberman Pinscher
PL732®[b]	3	0	2	3	1 Irish Setter 1 Labrador Retriever
XT612®[c]	3	0	2	2	1 Basset Hound 1 Golden Retriever 1 Labrador Retriever

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey

Blood Collection, Processing and Storage of Platelet Concentrates

Units of whole blood averaged 460 mL per phlebotomy. Total phlebotomy time did not exceed 15 minutes per draw. Once collected, each unit of blood was kept at room temperature and processed for separation into components immediately. Platelet concentrates were stored in a platelet incubator/rotator that was maintained at 22 º C; rotator motion was end over end at a speed of 6 rpm. The mean volume of platelet concentrates on Day 0 was 143 mL. The volume of each unit of platelet concentrate was maintained above 70 mL for the duration of the study.

Red Cell Contamination Rate and Platelet Yield

Red cell contamination rate and platelet yield were calculated on Day 0; the red cell contamination rate for all bags was 1.67% (Table 4). Visual color of the platelet concentrates ranged from straw to salmon. Mean platelet yield for all bags was 69.6% (Figure 1).

Table 4. Red Blood Cell Contamination Rates of Platelet Concentrates; n=5 for each type of container

Platelet Storage Container	Red Cell Contamination Rate
PL1240[a]	1.6%
PL732®[b]	1.7%
XT612®[c]	1.7%

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
For standard error values, see Appendix A_1
Red Cell Contamination Rate =(Red Cells in Platelet Concentrate / Red Cells in Whole Blood) x 100

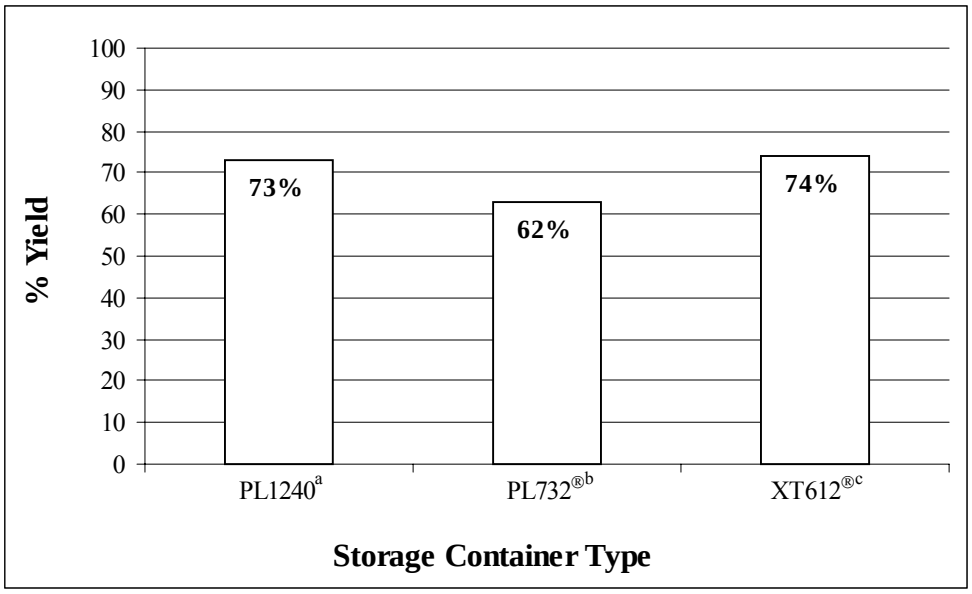

Figure 1. Mean Percent Platelet Yield of Platelet Concentrates; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
For standard error values, see Appendix A_2
Percent Platelet Yield = (Number of platelets in Platelet Concentrate/ Number of Platelets in Whole Blood) x 100

Biochemical Changes

The pH, pCO_2 and bicarbonate values of all platelet concentrates decreased during the 9-day room temperature storage (Figures 2-4). Values of these analytes obtained from samples analyzed on Day 3, 5, 7 and 9 were significantly different ($p < 0.001$) compared to samples analyzed on Day 0. For pH ($p < 0.0012$), pCO_2 ($p < 0.0139$) and bicarbonate ($p < 0.0024$), there was a significant difference between values obtained from bag XT612® and PL 732® on Day 9. In addition, bicarbonate values from bag XT612® and PL 732® were significantly different on Day 5 ($p < 0.0026$) and Day 7 ($p < 0.0006$).

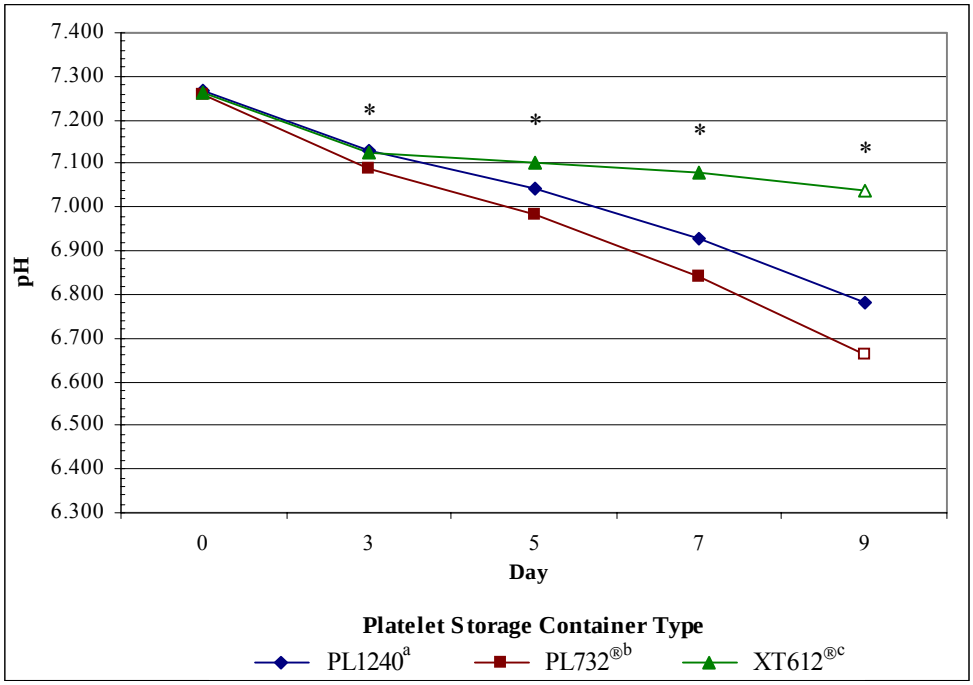

Figure 2. Mean pH Values of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
Significant difference between platelet storage container type denoted by open symbols
For mean and standard error values, see Appendix A_3

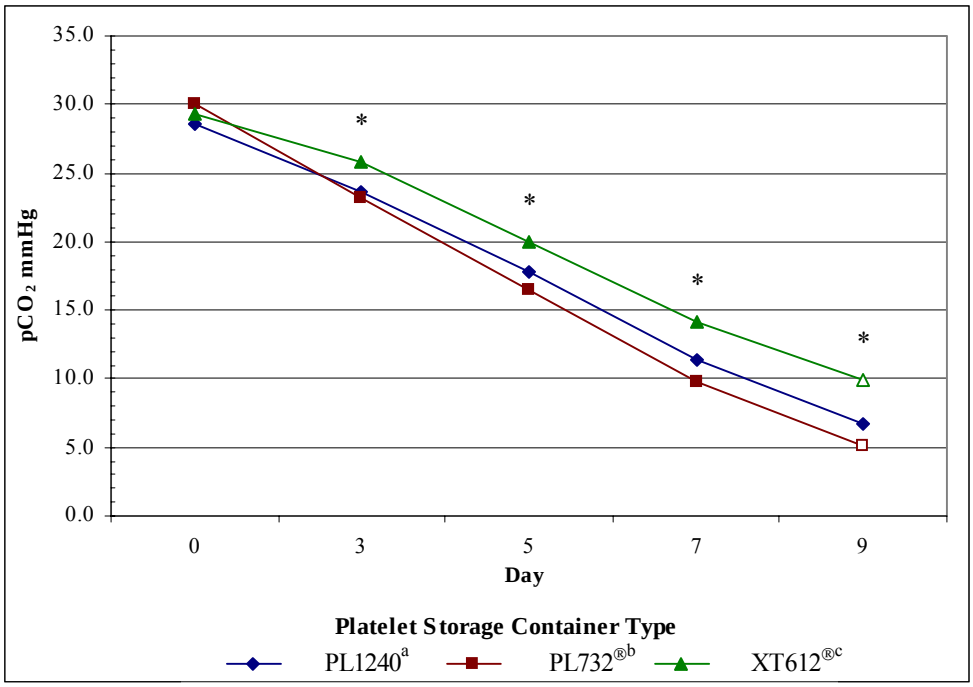

Figure 3. Mean Partial Pressure of Carbon Dioxide (pCO_2) Values in millimeters of mercury (mmHg) of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
Significant difference between platelet storage container type denoted by open symbols
For mean and standard error values, see Appendix A_4

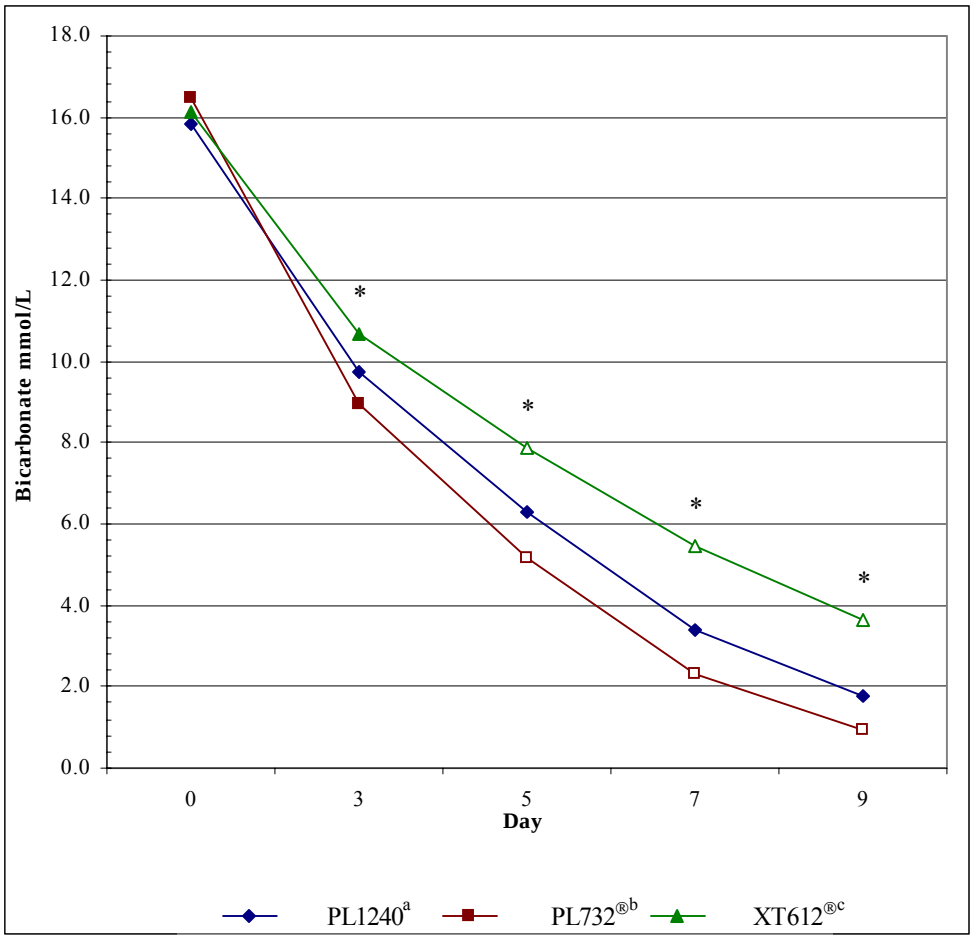

Figure 4. Mean Bicarbonate Values in millimoles per liter (mmol/L)of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
Significant difference between platelet storage container type denoted by open symbols
For mean and standard error values, see Appendix A_5

The partial pressure of oxygen values increased over 9 day room temperature storage (Figure 5). Differences were significant from all days when compared to Day 0 ($p < 0.001$) and there was a significant difference between bag XT612® and PL 732® on Day 3 ($p < 0.0032$) and between bag XT612® and PL 1240 on Day 9 ($p < 0.0140$)

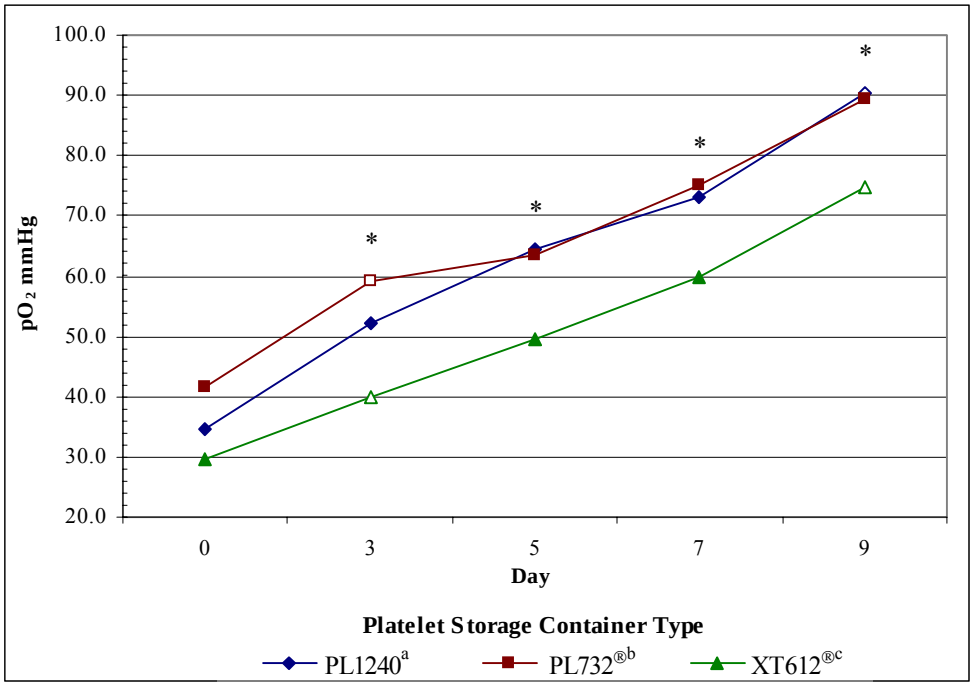

Figure 5. Mean Partial Pressure of Oxygen (pO_2) Values in millimeters of mercury (mmHg) of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
Significant difference between platelet storage container type denoted by open symbols
For mean and standard error values, see Appendix A_6

Glucose values decreased over the course of the 9 day storage period (Figure 6). Values obtained each day were significantly different ($p < 0.0001$) when compared to Day 0. There was a significant difference between glucose values obtained from bags XT612® and PL 732® for Day 7 ($p < 0.0013$) and Day 9 ($p < 0.0005$).

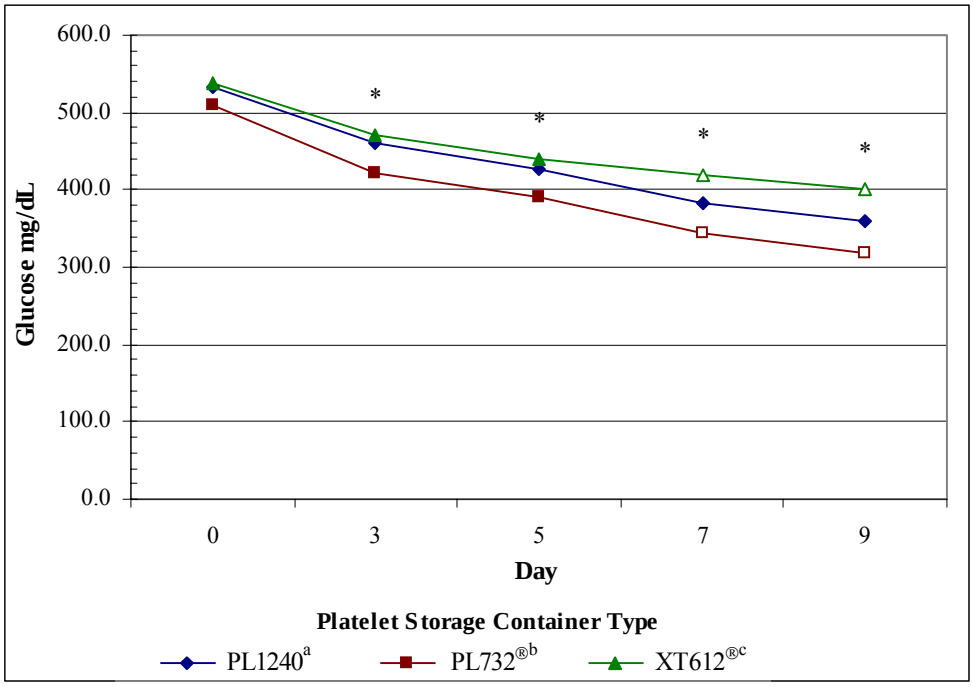

Figure 6. Mean Glucose Values in milligrams per deciliter (mg/dL)of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
Significant difference between platelet storage container type denoted by open symbols
For mean and standard error values, see Appendix A_7

Percent LDH Activity

Percent LDH activity in platelet concentrate plasma increased over the course of the 9 day study (Figure 7). Values obtained each day were significantly different ($p < 0.0001$) when compared to Day 0. Mean Day 9 LDH activity was 14.3% for all bags.

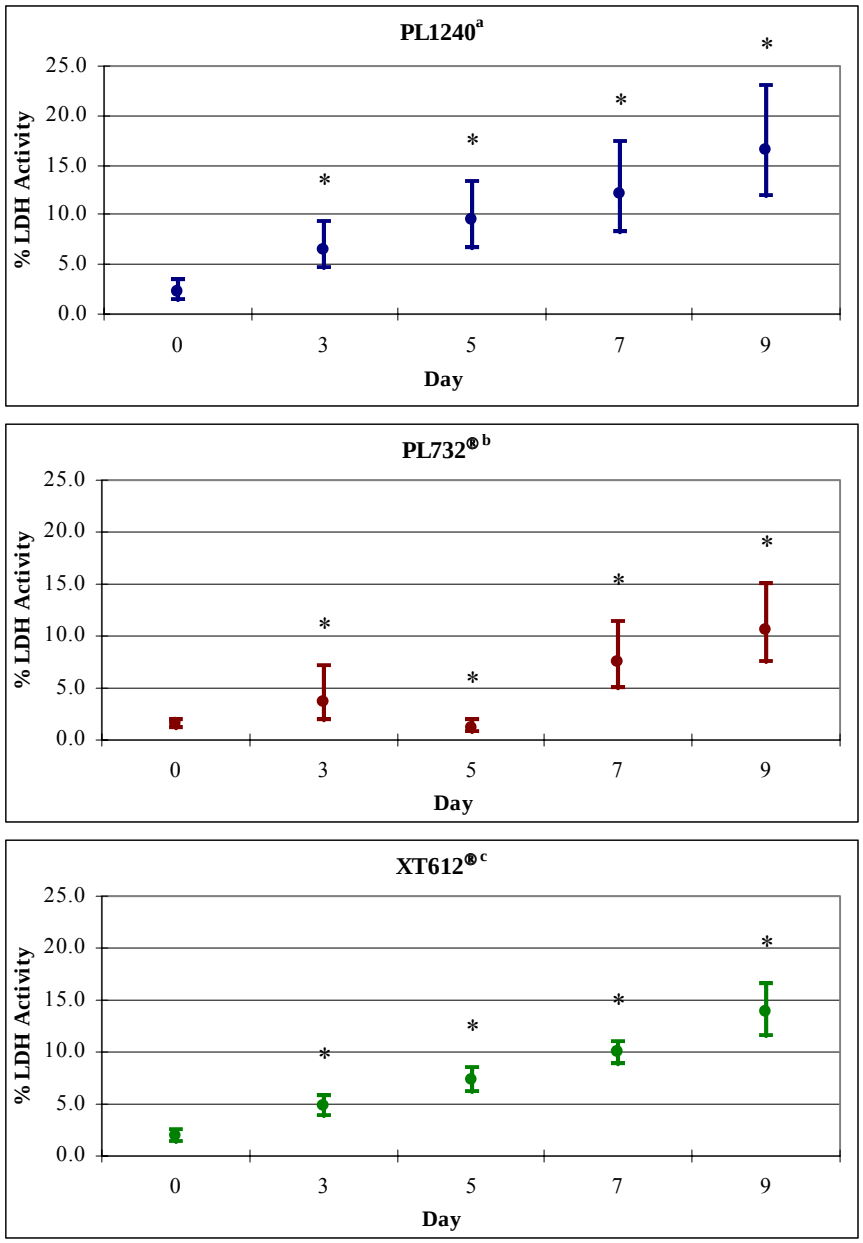

Figure 7. 95% Confidence Intervals for Percent LDH Activity (% LDH Activity)of Platelet Concentrates.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
For confidence interval values, see Appendix A_8

Platelet Count and Platelet Structural Changes

Platelet Count

The platelet count of all bag types decreased over the course of the 9 day study (Figure 8). Days 5, 7 and 9 were significantly different ($p < 0.001$) than Day 0. No significant difference between bag types was observed.

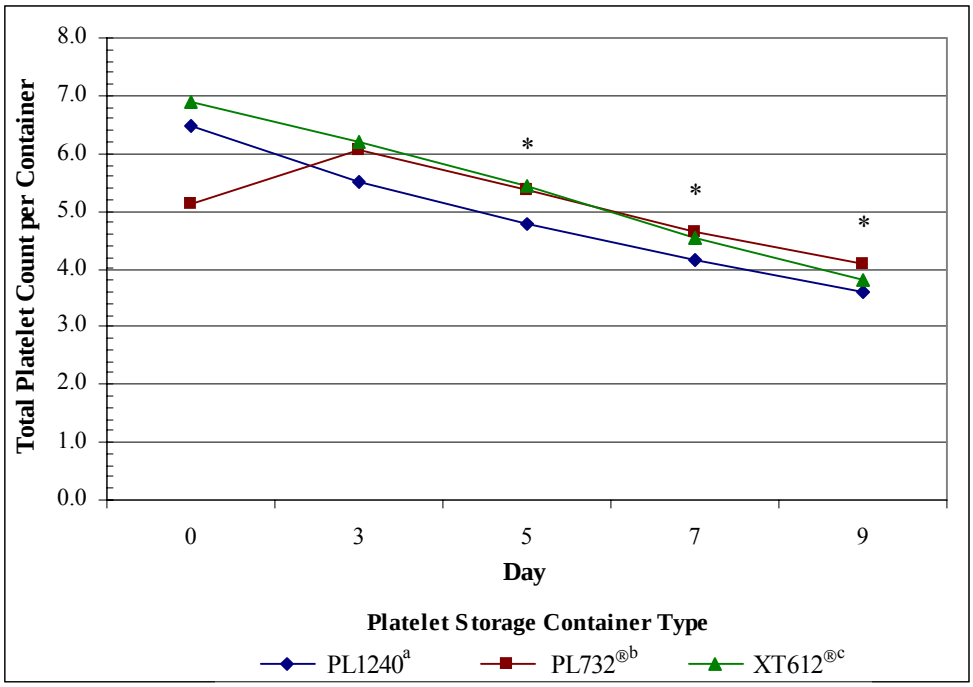

Figure 8. Total Platelet Count of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
For mean and standard error values, see Appendix A_9

Mean Platelet Volume

The average MPV from the units of whole blood was significantly different ($p < 0.001$) than the average MPV from the units of platelet concentrates on Day 0 for all bag types (Table 5). Significant difference between Day 0 and Day 3 ($p < 0.001$) and Day 0 and Day 5 ($p < 0.023$) was observed for all bag types of platelet concentrates for MPV (Figure 9). The MPV decreased at Day 3, and then increased from Day 5 through Day 9.

Platelet Container	Whole Blood MPV	PC Day 0 MPV
PL 1240[a]	7.3	7.8
PL 732®[b]	8.4	9.5
XT 612®[c]	7.3	8.2
All	7.6[d]	8.5[d]

Table 5. Average Mean Platelet Volume (MPV) in femtoliters of Whole Blood and Day 0 Platelet Concentrates (PC) n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
[d] Denotes significant difference between whole blood and platelet concentrates for all platelet storage containers
For standard error values, see Appendix A_{10}

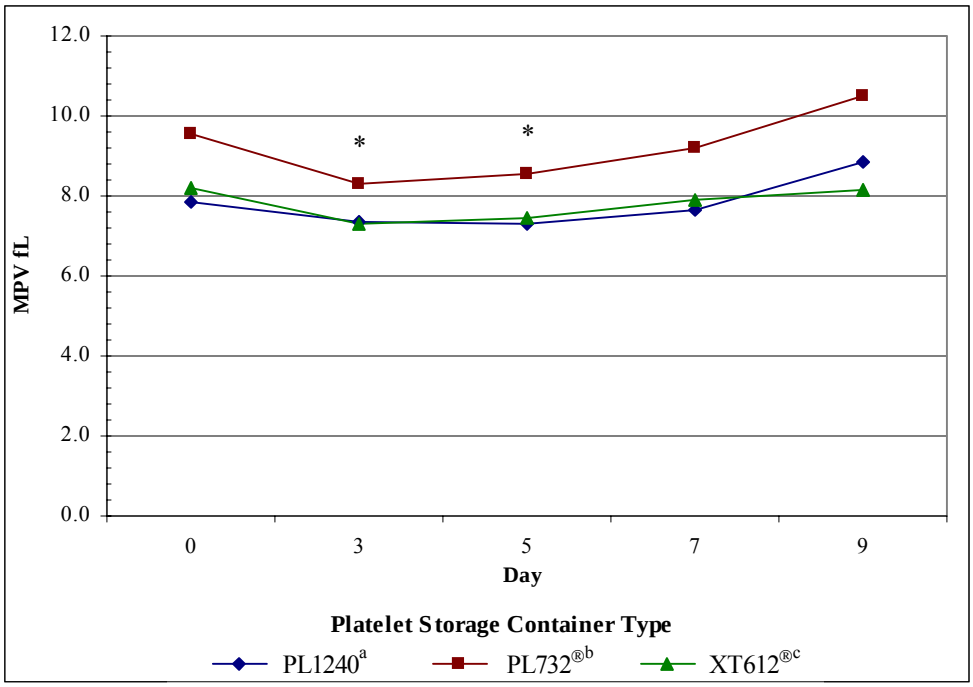

Figure 9. Average Mean Platelet Volume (MPV) in femtoliter (fL) of Platelet Concentrates by Day; n=5 for each type of platelet storage container.

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
* Denotes significant difference of all platelet storage containers from Day 0
For mean and standard error values, see Appendix A_{11}

Platelet Functional Changes

Percent Aggregation

No significant difference in percent platelet aggregation was observed between the three bag types (Figure 10) or comparatively between days. Day 0 values averaged 19.5% and decreased to 6.9% on Day 9.

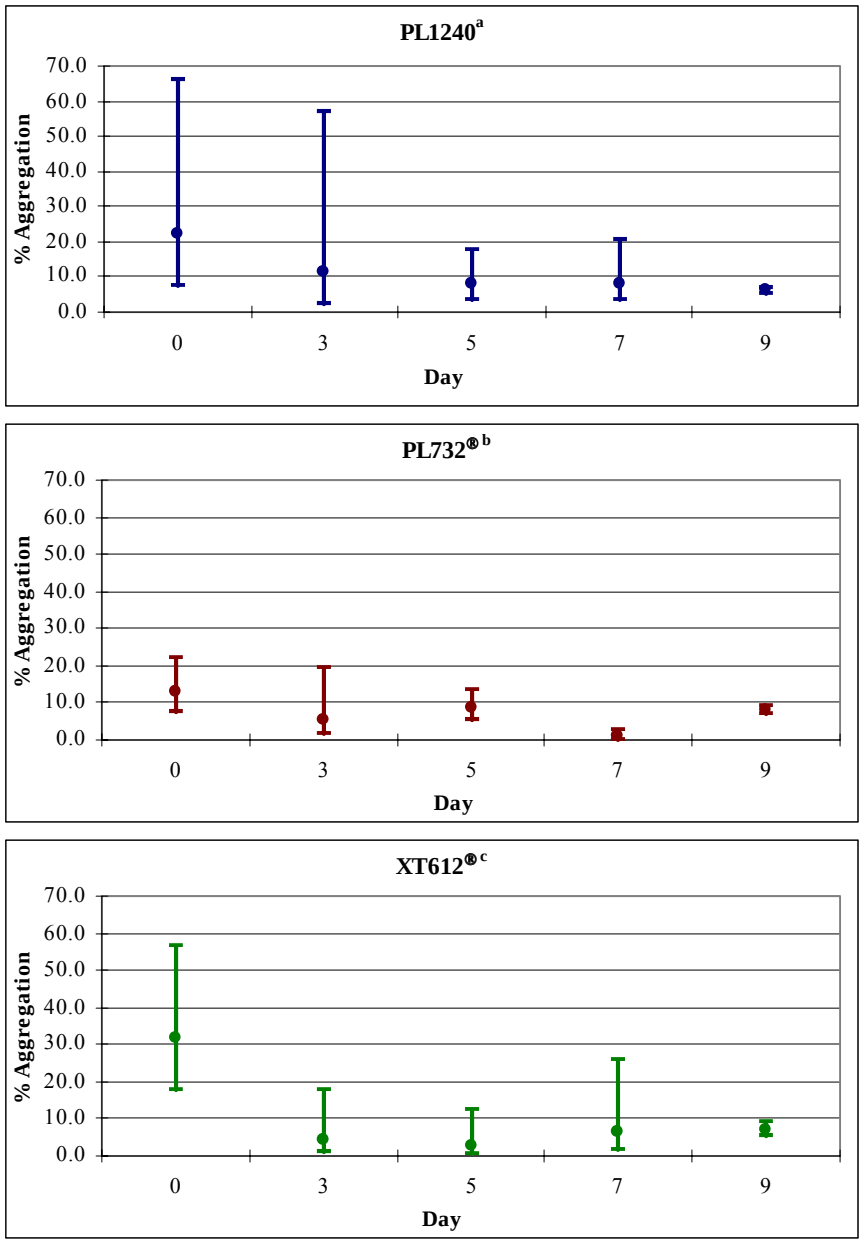

Figure 10. 95% Confidence Interval for Percent Platelet Aggregation (% Aggregation) of Platelet Concentrates

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois
[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey
For confidence interval values, see Appendix A_{12}

Hypotonic Shock Response

During the course of this study, only one 1240 unit recovered from hypotonic shock on Day 0. All other units reacted to both saline and water on all days, but recovery was not observed. Therefore, percent recovery was 0.

Microbiological Evaluation

Corynebacterium species was isolated from one PL 1240 bag beginning on Day 5. No other bacterial contamination was detected in any bag over the course of the study.

Discussion

Red Cell Contamination Rate

It is advantageous to provide a platelet product that contains a minimal number of red blood cells, since red cells may induce platelet activation[14] and may cause sensitization to red cell antigens once the product is transfused.[53] The task of extracting platelets from centrifuged whole blood without red cell contamination is challenging, as red cells lie just below the platelet layer. Centrifuge calibration provides a method to maximize platelet yield and minimize red cell contamination.[36]

The AABB specifies that levels of red cell contamination should be acceptable; yet the AABB does not define this value.[36] Previous reports have cited target values of less than one percent.[6] The American Red Cross bases its red cell contamination rate on visual inspection. Platelet concentrates that are light straw or light pink are acceptable, those that are pink, salmon or cherry in color are not acceptable for use. (personal communication, L. Snidow, 7/24/01) Thus, red cell numbers and concentrate color should be considered when selecting optimum centrifuge speed and time.

For use in this study, centrifuge calibration was performed and light spin was established at 3369 x g for three minutes duration. During centrifuge calibration, this protocol produced a 79.5% platelet yield in platelet rich plasma and a 1.46% red cell contamination rate. The platelet rich plasma was straw in color. Red cell contamination rate observed for the 15 platelet concentrates produced in the body of this study was 1.67% and colors ranged from straw to orange. Red cell contamination rate was consistent between bag types. Red cell counts from units passing visual inspection ranged from 212,000 – 434,000/μL while red cell counts from units that did not pass visual inspection ranged from 223,000 – 460,000/ μL. Platelet poor plasma samples from those units which did not meet the visual criterion for color were inspected for hemolysis; no hemolysis was evident. This study found discordance between red cell contamination

rates and visual inspection. Thus, visual inspection for red cell contamination in canine platelet concentrates appears to be of limited use. In the units passing visual inspection, the lowest red cell count obtained was 212,000/μL and corresponds to a red cell contamination rate of 1.04%. This value approximates red cell contamination rates of a previous study[6] and suggests that target red cell contamination rates of approximately 1% should be the goal for future preparations of platelet concnetrates. Further studies in varying centrifugal time and force may eliminate the problem of excessive red cell contamination as found in this study.

Platelet Yield and Platelet Count

The AABB states that at least 75% of all platelet concentrates contain a minimum of 5.5 X 10^{10} platelets per bag. In this study, 60% of all platelet concentrates contained greater than this number. Included in this percentage are two PL 732® bags that did not meet this criterion until Day 3, since on Day 3, the platelet count from these two PL 732® bags exceeded both the platelet count from Day 0 and the AABB recommendation. In this study, samples for *in vitro* studies were extracted on Day 0 after a one hour resting period (to promote platelet disaggregation) and a two hour resuspension period. Visible aggregates were present in the aforementioned two PL 732® bags on Day 0, suggesting that disaggregation was not complete upon sampling for *in vitro* studies. This was supported by the fact that by Day 3, platelet content of these two PL 732® bags approached the values obtained from the other two bag types on Day 3. This finding warrants further investigation and may prohibit use of this bag for collection and storage of canine platelet concentrates.

Each bag type was equally represented within the 40% of the platelet concentrate units that did not meet the 5.5 X 10^{10} platelets per bag criterion. The average platelet content for these bags was 4.5 X 10^{10}, with a range from 3.99-5.15 X 10^{10} per bag. The average number of platelets for all bags on Day 0 was 6.16 X 10^{10} per bag. This finding falls between platelet values reported in two previous investigations involving canine platelet concentrates, which reported average platelet content of 8.0 X 10^{10} [5] and 5.92 X 10^{10} [6] per

bag. Throughout storage, platelet numbers decline due to cell death and lysis; this was observed in this study, as the average platelet count per bag decreased by 40% by Day 9.

Indicators of Metabolic Activity of Platelet Concentrates

As previously discussed, metabolism of glycogen and glucose for ATP production continues during storage of platelet concentrates. Glycogen is present intracellularly and the residual plasma within the platelet concentrate contains sufficient glucose for 5 day storage of human platelets.[25] A previous study of human platelet concentrates stored for 5 days reported a mean glucose concentration of 288 mg/dL on Day 4 (Day 5 was not tested), and concluded that this glucose concentration did not appear to affect *in vivo* survival.[54] In a previous study of canine platelet concentrates, mean glucose concentrations ranged from 504.5 mg/dL on Day 0 to 270.3 mg/dL on Day 7.[6] Comparatively, mean glucose concentrations found in this study were higher, as mean glucose concentrations for all bag types ranged from 527 mg/dL on Day 0 to 360 mg/dl on Day 9. The lowest mean glucose concentration was 319 mg/dL in bag type PL 732® on Day 9. Based on these findings, it appears that glucose concentrations in canine platelet concentrates used in this study were maintained at acceptable concentrations to support platelet survival in all bag types throughout 9 day storage.

Over this 9 day study, pO_2 values increased while pCO_2 and bicarbonate values decreased. This pattern is a documented effect of the storage lesion of platelet concentrates using second generation storage containers.[25-27] The partial pressure of oxygen in room air (160 mmHg) is greater than the partial pressure of oxygen within the platelet storage container; conversely, the partial pressure of carbon dioxide in room air (0.25 mm Hg) is less than the partial pressure of carbon dioxide within the platelet storage container. Plasticizers used in second generation storage containers facilitate the influx of oxygen and the efflux of carbon dioxide.[25] This exchange is necessary for two reasons. First, oxygen is consumed through cellular respiration and must be replenished for respiration to continue during platelet storage. Secondly, lactic acid, a byproduct of glycolysis, is ultimately reduced to carbon dioxide by bicarbonate. Without efflux of

carbon dioxide, lactic acid accumulates which results in a decrease in the pH of the platelet concentrate. Without gas exchange, oxygen levels would diminish and carbon dioxide levels would increase within the storage container. This was not observed in this study; therefore, gas exchange of all three bag types was appropriate to aid in maintenance of platelet viability.

In this study, platelet storage container XT612® demonstrated superior maintenance of bicarbonate concentration beginning on Day 5 when compared to PL 732®; difference from PL 1240 was not detected. However, bicarbonate, pCO_2 and pO_2 concentrations did not appear to adversely affect pH, which was maintained above 6.2 during 9 day storage. Based on these findings, all bag types appear to be suitable for 9 day storage of canine platelet concentrates in regard to gas exchange.

The pH of all units of platelet concentrates produced in this study ranged from a mean value of 7.625 on Day 0 to 6.827 on Day 9. Over the course of the study, the lowest pH value obtained was 6.192 on Day 9 from a PL 1240 container. Otherwise, all other pH values adhered to the FDA and AABB standard of a minimum pH of platelet concentrates of greater than or equal to 6.2. Thus, the pH of canine platelet concentrates stored for 9 days in all bag types appears to be adequate to support platelet survival.

LDH is a cytosolic enzyme and its presence in the plasma of platelet concentrates has been demonstrated as a marker of platelet lysis in both humans and dogs.[6,55,56] Studies using human platelet concentrates reported acceptable percent LDH activity ranging from 5.5 – 20% during 5 day storage.[26,55,57] Mean values from canine platelet concentrates have been reported as 8% on Day 5 and Day 7 of storage.[5] This study found mean values of 7.7% LDH activity on Day 5, 10.4% LDH activity on Day 7 and 14% LDH activity on Day 9. Although these percent LDH activity values were higher than values previously reported for canine platelet concentrates, LDH activity was well within the range reported

as acceptable for 5 day human platelet concentrate storage. This implies that excessive numbers of canine platelets are not lysed during 9 day storage in all three bag types.

Platelet Structural Changes

The average MPV derived from whole blood samples from all units used in this study prior to processing was 7.63 fL. Once processing occurred, the average value of MPV for all platelet concentrates increased to 8.5fL. This change is indicative of an increase of platelet volume and subsequent shape change.[39] This was possibly caused by platelet activation during component preparation, as platelet activation in platelet concentrates has been associated with increased MPV.[37] This is supported by data from Day 3 MPV values, which equaled those found in whole blood prior to processing, suggesting that platelets were capable of returning to normal volume and shape via osmotic equilibrium. Average MPV values began to increase again by Day 5, suggesting that the stored platelets were unable to maintain osmotic equilibrium and subsequently lost discoid shape. Based on this finding, it appears that platelets in concentrates began to deteriorate by Day 5 in all bag types.

Platelet Functional Changes

Platelet Aggregation

As platelets are collected, processed and stored, platelet activation occurs.[14] Activation includes many changes within the platelet, including, but not limited to, extension of pseudopodia, concentration of organelles and changes in the state of receptors.[12] Platelet aggregation studies are one of the most common methods used to evaluate stored platelets from humans[8,58], aside from pH and platelet count.[8] The ability of platelet concentrates to respond to platelet agonists is a test of platelet functionality and lack of aggregation from these stimuli suggests decreased platelet viability.[59]

Selection of paired agonists used in this study were based on human and canine studies[5,43], as paired agonists have been shown to demonstrate a greater response than

agonists used singly. Collagen was selected since *in vivo*, damaged endothelial surfaces expose subendothelial collagen, which with the addition of ADP, activates platelets. Only one set of paired agonists was tested, since platelet aggregation studies consumed a considerable amount of sample for laboratory analysis and volume depletion of the platelet concentrate over the nine day study was a concern.

No significant differences in percent platelet aggregation were observed between the three bag types. Percent platelet aggregation values found in this study are considerably lower than those previously reported for canine platelet concentrates[5], which reported 50% aggregation on Day 0, 40% aggregation on Day 5 and 20% aggregation on Day 7. Human platelet concentrates have been reported as 78% aggregation on Day 0, decreasing to 29% by Day-5.[12] The results of the previous studies suggest that aggregatory response of canine platelet concentrates produced in this study was compromised beginning Day 0. This may be attributed to the fact that canine platelet concentrates produced in this study were contaminated by red cells at a rate slightly in excess of 1%, and it has been reported that platelet concentrates contaminated by excess red cells demonstrate a diminished aggregatory response.[6]

Hypotonic Shock Response

This study did not observe a canine platelet response to hypotonic shock. The only other study of hypotonic shock response in canine platelet concentrates also failed to observe recovery similar to that of human platelet concentrates.[6] It is possible that canine platelets in concentrates do not respond similarly to human platelet concentrates.[6] Based on this and the previous study, hypotonic shock response appears to be of no use for evaluating the storage lesion of canine platelet concentrates.

Bacterial Contamination

In this study, closed blood collection systems were used in order to minimize potential bacterial contamination of platelet concentrates. However, a sample coupling device was spiked into the platelet concentrate container on Day 0 in order to provide a port to obtain

specimens for laboratory analysis. This practice violates blood bank protocol for banked platelets, as any opening of the closed system would mandate a 4 hour product shelf life.[60]

All platelet concentrates that were harvested in this study were negative for bacterial growth with the exception of one PL 1240 unit which was found to contain *Corynebacterium* species beginning on Day 5. This bacterium could have been introduced into the unit during phlebotomy, as *Corynebacterium* species[61] could have been aspirated through the needle. This bacterium also resides as normal skin flora of humans[62], and may have been introduced into the unit by the investigator when obtaining specimens for laboratory analysis. Using the collection, storage and sampling methods of this study, bacterial contamination may be more likely in bag type PL 1240 as compared to PL 732® and XT612®. However, additional studies involving bacteriological evaluation of stored platelet concentrates which have not been multi-sampled are necessary to establish superiority of maintaining sterility for any particular type of platelet storage bag.

Conclusion

This study indicates that canine platelet concentrates may be harvested by differential centrifugation of whole blood. *In vitro* tests monitoring metabolic function indicate that canine platelet concentrates appear to be viable for nine days when stored in three second generation storage containers. All platelet concentrates produced in this study maintained sterility throughout 5 day storage and platelet structure began to deteriorate by Day 5. While there was no consensus of data supporting the use of one platelet storage container based on superior outcome of *in vitro* tests, these data support the finding of a previous study and concur that canine platelet concentrates stored at room temperature using continuous agitation are viable for at least 5 days. However, due to poor performance during platelet aggregation testing, questions remain about the functionality of the platelet concentrates produced by the protocol established by this study.

Future Considerations

Recommendations for use of canine platelet concentrates cannot be made from the results of this study alone since further research should first be performed to determine platelet viability once transfused. In human medicine, the response to platelet transfusion is assessed by observing whether bleeding ceases and by measuring the pre- and posttransfusion platelet counts. The posttransfusion platelet count is measured between 10-60 minutes after completion of the platelet transfusion and is expressed as the corrected count increment (CCI). The CCI corrects for the number of platelets infused and the blood volume of the recipient as follows[63]:

$$\text{CCI at 1 hour} = \frac{(\text{Platelet count}_{post} - \text{Platelet count}_{pre}) \times \text{Body Surface Area in square meters}}{\text{Number of platelets transfused in multiples of } 10^{11}}$$

Studies involving transfusion of platelet concentrates to dogs and measuring this response must be performed before recommendations for routine use of this blood component can be made.

Manuscript Footnotes

[a] PL 1240 plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois

[b] PL 732® plastic Transfer Pack, Baxter Healthcare Corporation, Deerfield, Illinois

[c] XT612® 5-day platelet bag, Terumo Medical Corporation, Somerset, New Jersey

[d] Blood Collection Chamber, Animal Blood Bank, Dixon, California

[e] Hematron Dielectric Sealer, Baxter Healthcare Corporation, Deerfield, Illinois

[f] Sorvall RC 3B Plus, Kendro Laboratory Products, Newton, Connecticut

[g] Plasma Extractor, Baxter Healthcare Corporation, Deerfield, Illinois

[h] Mettler-Toledo Ag Scale, Greifensee, Switzerland

[i] Sampling Site Coupler, Terumo Medical Corporation, Somerset, New Jersey

[j] PC400 Platelet Inucbator/Rotator, Helmer Incorporated, Noblesville, Indiana

[k] Rapidlab™ 348, Bayer Corporation, Medfield, Massachusetts

[l] Cell-Dyn 3700, Abbott Laboratories, Incorporated, Abbott Park, Illinois

[m] Olympus AU400, Olympus of America, Incorporated, Melville, New York

[n] Triton-X 100, Sigma Chemical, St. Louis, Missouri

[o] Immufuge® II, Baxter Healthcare Corporation, Deerfield, Illinois

[p] Prostaglandin E_1, Sigma Chemical, St. Louis, Missouri

[q] Platelet Aggregometer Model 490-2D, Chrono-Log Corporation, Havertown, Pennsylvania

[r] ADP reagent, Chrono-Log Corporation, Havertown, Pennsylvania

[s] Collagen reagent, Chrono-Log Corporation, Havertown, Pennsylvania

[t] Phosphate Buffered Saline, Sigma Chemical, St. Louis, Missouri

[u] Sterile Water for Irrigation, Baxter Healthcare Corporation, Deerfield, Illinois

[v] Brain Heart Infusion, Remel Microbiology Products, Richmond, Virginia

[w] Blood Agar, Remel Microbiology Products, Richmond, Virginia

[x] MacConkey Agar, Remel Microbiology Products, Richmond, Virginia

[y] Brucella Agar, Remel Microbiology Products, Richmond, Virginia

[z] SAS, v.8.02, SAS Institute, Cary, North Carolina

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Appendix A: Mean and Standard Error Values by Analyte

A_1 Red Cell Contamination Rate in Percent

Platelet Container	Mean	Standard Error
PL 1240	1.6	0.721
PL 732	1.7	0.359
XT 612	1.7	0.417

A_2 Percent Platelet Yield in Percent

Platelet Container	Mean	Standard Error
PL 1240	73.3	7.748
PL 732®	61.6	7.206
XT 612®	74.0	6.537

A_3 pH

Day	Platelet Container	Mean	Standard Error
0	PL 1240	7.265	0.0132
	PL 732®	7.260	0.0132
	XT 612®	7.263	0.0121
3	PL 1240	7.128	0.0642
	PL 732®	7.088	0.0319
	XT 612®	7.125	0.0363
5	PL 1240	7.042	0.0807
	PL 732®	6.983	0.0456
	XT 612®	7.102	0.0416
7	PL 1240	6.927	0.1080
	PL 732®	6.839	0.0636
	XT 612®	7.081	0.0480
9	PL 1240	6.779	0.1661
	PL 732®	6.661	0.0877
	XT 612®	7.040	0.0609

A_4 pCO_2 in mmHg

Day	Platelet Container	Mean	Standard Error
0	PL 1240	28.6	1.379
	PL 732®	30.1	1.156
	XT 612®	29.3	1.019
3	PL 1240	23.6	2.334
	PL 732®	23.2	1.449
	XT 612®	25.8	1.526
5	PL 1240	17.7	1.287
	PL 732®	16.5	1.547
	XT 612®	19.9	1.603
7	PL 1240	11.4	0.645
	PL 732®	9.8	1.392
	XT 612®	14.2	1.604
9	PL 1240	6.6	0.785
	PL 732®	5.1	1.004
	XT 612®	9.9	1.518

A_5 Bicarbonate in mmol/L

Day	Platelet Container	Mean	Standard Error
0	PL 1240	15.8	0.368
	PL 732®	16.5	0.698
	XT 612®	16.1	0.301
3	PL 1240	9.7	0.584
	PL 732®	9.0	0.886
	XT 612®	10.7	0.721
5	PL 1240	6.3	0.748
	PL 732®	5.2	0.723
	XT 612®	7.9	0.761
7	PL 1240	3.4	0.752
	PL 732®	2.3	0.478
	XT 612®	5.5	0.810
9	PL 1240	1.8	0.604
	PL 732®	0.9	0.222
	XT 612®	3.6	0.788

A_6 pO_2 in mmHg

Day	Platelet Container	Mean	Standard Error
0	PL 1240	34.5	4.127
	PL 732®	41.4	3.272
	XT 612®	29.5	4.154
3	PL 1240	52.3	5.194
	PL 732®	59.1	4.526
	XT 612®	39.8	4.915
5	PL 1240	64.6	6.745
	PL 732®	63.4	2.667
	XT 612®	49.5	4.819
7	PL 1240	73.0	6.035
	PL 732®	74.9	2.700
	XT 612®	59.8	3.802
9	PL 1240	90.5	4.434
	PL 732®	89.5	3.927
	XT 612®	74.6	4.657

A_7 Glucose in mg/dL

Day	Platelet Container	Mean	Standard Error
0	PL 1240	534	15.09
	PL 732®	510	6.03
	XT 612®	539	8.98
3	PL 1240	459	18.26
	PL 732®	422	7.07
	XT 612®	472	10.87
5	PL 1240	426	17.63
	PL 732®	392	8.12
	XT 612®	441	23.58
7	PL 1240	383	21.20
	PL 732®	344	9.32
	XT 612®	419	11.89
9	PL 1240	360	28.98
	PL 732®	319	12.34
	XT 612®	402	12.84

A_8 95% Confidence Intervals for Percent LDH Activity

Day	Platelet Container	Mean	lower	upper
0	PL 1240	2.22	1.47	3.33
	PL 732®	1.55	1.23	1.96
	XT 612®	1.88	1.41	2.50
3	PL 1240	6.48	4.54	9.25
	PL 732®	3.70	1.94	7.05
	XT 612®	4.75	3.88	5.82
5	PL 1240	9.49	6.75	13.32
	PL 732®	1.22	0.82	1.83
	XT 612®	7.26	6.17	8.54
7	PL 1240	12.03	8.32	17.39
	PL 732®	7.57	5.03	11.38
	XT 612®	9.91	8.91	11.02
9	PL 1240	16.56	11.97	22.91
	PL 732®	10.57	7.42	15.06
	XT 612®	13.82	11.60	16.45

A_9 Platelet Count X 10^{10}/uL

Day	Platelet Container	Mean	Standard Error
0	PL 1240	6.46	0.860
	PL 732®	5.14	0.556
	XT 612®	6.88	1.053
3	PL 1240	5.49	0.893
	PL 732®	6.04	0.749
	XT 612®	6.21	0.743
5	PL 1240	4.77	0.801
	PL 732®	5.37	0.752
	XT 612®	5.45	0.657
7	PL 1240	4.15	0.779
	PL 732®	4.64	0.729
	XT 612®	4.53	0.560
9	PL 1240	3.59	0.718
	PL 732®	4.08	0.768
	XT 612®	3.80	0.478

A_{10} MPV in fL

	Platelet Container	Mean	Standard Error
Whole Blood	PL 1240	7.3	0.292
	PL 732®	8.4	0.685
	XT 612®	7.3	0.261
Day 0	PL 1240	7.8	0.418
	PL 732®	9.5	0.799
	XT 612®	8.2	0.352

A_{11} MPV in fL

Day	Platelet Container	Mean	Standard Error
0	PL 1240	7.8	0.418
	PL 732®	9.5	0.799
	XT 612®	8.2	0.352
3	PL 1240	7.3	0.353
	PL 732®	8.3	0.561
	XT 612®	7.3	0.254
5	PL 1240	7.3	0.380
	PL 732®	8.6	0.445
	XT 612®	7.5	0.227
7	PL 1240	7.7	0.579
	PL 732®	9.2	0.599
	XT 612®	7.9	0.281
9	PL 1240	8.9	1.087
	PL 732®	10.5	0.978
	XT 612®	8.1	0.291

A_{12} 95% Confidence Interval for Percent Aggregation

Day	Platelet Container	Mean	lower	upper
0	PL 1240	22.32	7.57	65.76
	PL 732®	12.70	7.32	22.05
	XT 612®	31.52	17.66	56.27
3	PL 1240	11.57	2.34	57.10
	PL 732®	5.37	1.50	19.24
	XT 612®	4.36	1.06	17.93
5	PL 1240	7.87	3.55	17.47
	PL 732®	8.57	5.46	13.46
	XT 612®	2.88	0.66	12.64
7	PL 1240	8.00	3.14	20.36
	PL 732®	1.00	0.00	2.54
	XT 612®	6.43	1.61	25.71
9	PL 1240	6.00	5.40	6.67
	PL 732®	8.00	7.20	8.89
	XT 612®	6.928	5.20	9.24

Carolyn Anne Sink

EDUCATION	Virginia Polytechnic Institute and State University, Blacksburg, Virginia MS 2002 Veterinary Medical Science Radford University, Radford Virginia BS 1981 Medical Technology
EXPERIENCE	Clinical Laboratory Supervisor *Virginia-Maryland Regional College of Veterinary Medicine* Blacksburg, Virginia 1989-present Plans, organizes, directs and coordinates the technical and administrative functions of Clinical Laboratory Services of the Veterinary Teaching Hospital comprised of Laboratory Central Receiving, Clinical Pathology, Immunology , Microbiology, Parasitology and Blood Bank. Directly maintains equipment in the Clinical Pathology Laboratory. Performs technical duties of a medical technologist in Laboratory Services, primarily in Blood Bank and Clinical Pathology. Trains and supervises emergency and on-call student wage employees who provide 24/7 coverage for the Clinical Pathology Laboratory Medical Technologist *Saint Alban's Psychiatric Hospital* Radford, Virginia 1988-1989 (part-time) Collected venous and capillary blood specimens. Performed routine hematology, chemistry, urinalysis and substance of abuse testing. Evaluated and verified test results. Identified and solved technical and instrumentation problems. Maintained appropriate records and performed clerical duties related to the laboratory. Medical Technologist *Montgomery Regional Hospital* Blacksburg, Virginia 1987-1988 Collected venous and capillary blood specimens. Performed routine and complex hematology, coagulation, chemistry, urinalysis and limited microbiology. Evaluated and verified test results. Identified and solved technical and instrumentation problems. Maintained appropriate records and performed clerical duties related to the laboratory. Blood Bank/Phlebotomy Supervisor *Lewis Gale Hospital* Salem, Virginia 1984-1987 Planned, organized, directed and coordinated the technical and administrative functions of Blood Bank and Phlebotomy Sections within Laboratory Services. Performed technical duties of a medical technologist in Blood Bank. Hired and trained personnel for all shifts.
PROFESSIONAL ORGANIZATIONS	American Society of Clinical Pathologists MT(ASCP) 142278 American Society for Veterinary Clinical Pathology
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