
by

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Abstract

The purpose of this investigation was to develop and evaluate a high-speed, continuous-flow, automated plasmapheresis procedure for the collection of equine plasma according to current good manufacturing practices. Once a safe and reliable method was achieved, the plasmapheresis procedure was used to perform serial donations. The effect of serial plasmapheresis on total plasma protein and total IgG concentrations was evaluated throughout the investigation.

Adaptations were made to human-model automated plasmapheresis instruments and sterile collection sets, which allowed for dual-instrument, continuous-flow operation. Equine donors were connected to the modified collection sets via jugular vein catheters. The instruments extracted whole blood, infused an anticoagulant, fractionated the anticoagulated whole blood, harvested the plasma and returned the concentrated blood cells to the donors.

The procedures lasted between 3 to 6 hours in length and yielded approximately 20L of plasma per 900kg horse. During the investigation, 3,240 plasmapheresis donations were performed, 143 different horses were plasmapheresed and more than 50,000L of plasma were collected. The majority of donors tolerated the procedure and remained calm during plasmapheresis. Adverse events associated with the procedure were minor, occurred with an extremely low frequency or were eliminated after making adjustments to the technique.
The described plasmapheresis procedure was used to harvest 22ml of plasma per kg donor body weight at 14-day intervals. Donors experienced a statistically significant ($p < 0.05$) decrease in both total plasma protein and total IgG concentrations during the investigation. However, the decreases in concentration were not physiologically significant. Neither total protein nor total IgG concentrations were below the reference interval at the time of donation. Additionally, the concentrations did not decrease further with serial donations, but stabilized at a reduced concentration or returned to baseline. Despite the decreases in IgG concentration, the donors did not demonstrate an increase in disease incidence. These results indicate that the described serial plasmapheresis protocol allowed time for donor horses to replace lost plasma proteins and IgG.

A safe and reliable method for automated equine plasmapheresis was achieved. The collection of 22ml of plasma per kg donor body weight at 14-day intervals did not cause sustained total protein or IgG depletion in the donors.
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CHAPTER I

Literature Review

**Blood:**

Blood is a connective tissue consisting of solid elements suspended in a nonliving liquid matrix. In human beings, the cellular elements account for approximately 40% of whole blood and include erythrocytes, leukocytes and platelets (Guyton A, Hall J, 2000). Plasma, the liquid matrix, consists of greater than 90% water. Mammalian blood functions as a transportation system to move dissolved gases throughout the body; to deliver nutrients from the gastrointestinal tract to the liver; to carry hormones from glands to target tissues; and to take waste products to sites for excretion. Blood also plays an important role in the innate and adaptive immune responses. Additionally, blood assists in the regulation of pH, body temperature and electrolyte levels (Akers R, Denbow D, 2008).

**Cellular Components of Blood:**

Similar to human beings and other mammals, the cellular elements of equine blood consist of erythrocytes, leukocytes and platelets. The erythrocytes, or red blood cells, contain hemoglobin and function in gas transportation (Akers R, Denbow D, 2008, Guyton A, Hall J, 2000). Equine red blood cells are relatively small and show a tendency for prominent rouleaux formation, which causes the cells to rapidly separate from the plasma (Ziemer E, Bloom J, 1999). As in other mammalian erythrocytes, equine erythrocytes lack nuclei and organelles. However, small, round, basophilic, nuclear
remnants known as Howell-Jolly bodies are occasionally seen in equine erythrocytes. The presence of these red cell inclusions is normal and does not indicate a responsive anemia (Morris D, 2002). In fact, equine erythrocytes remain in the bone marrow until hemoglobin synthesis is complete, even when erythropoiesis is intense. Therefore, reticulocytosis and other peripheral blood signs of bone marrow regeneration are rarely observed in horses (Morris D, 2002, Ziemer E, Bloom J, 1999). The packed cell volume (PCV) is highly variable in horses and may be attributable to the unique equine spleen. Horses have a highly innervated muscular spleen that may contain up to one third of the red cell mass in health. With adrenergic stimulation, the spleen contracts and releases its reservoir of erythrocytes into circulation, causing the PCV to increase by as much as 50% (Morris D, 2002). The red cell numbers also vary with horse breeds. Draft and pony breeds may have PCV values as low as 24% in health, while racing Thoroughbreds may have a PCV near 50% (Ziemer E, Bloom J, 1999). Seven independent red blood cell groups or systems have been defined in horses with over 30 different erythrocyte alloantigens identified. Alloantigens Aa and Qa are the most immunogenic and most clinically significant (Blackmer J, Parish S, 2002, Morris D, 1999).

As in other mammals, horses possess five different types of mature white blood cells, which are categorized as either polymorphonuclear (PMN) leukocytes or mononuclear leukocytes (Morris D, 2002). Neutrophils, eosinophils and basophils account for the PMN leukocytes, while the monocytes and lymphocytes are classified as mononuclear leukocytes. Neutrophils are the most abundant white blood cell in horses, accounting for greater than half of the leukocytes in a normal adult horse. Neutrophils phagocytize and destroy invading pathogens. Eosinophils play an important role in
controlling parasitic infections. Basophils, which are the least numerous leukocyte in equine blood, elicit immediate hypersensitivity reactions through secretion of vasoactive mediators. Monocytes circulate in the equine blood for 1-3 days and then enter tissues where they mature into macrophages. Tissue macrophages defend against pathogens, remove diseased or effete cells, remodel tissues and secrete an array of factors with a diverse range of biological importance (Latimer K, 1999, Morris D, 2002). The lymphocytes are classically divided into T cells (thymus derived lymphocytes) and B cells (bone marrow derived lymphocytes). These white blood cells are responsible for mounting the adaptive immune responses. Cell-mediated immunity is governed by the T lymphocytes and is directed against microbes that survive within phagocytes or infected nonphagocytic cells. Humoral immunity is mediated by antibodies secreted from the B lymphocytes and targets extracellular microbes or microbial toxins (Abbas A, Lichtman A, 2005).

Platelets account for the final formed element in equine blood. The platelets are actually cellular fragments, whereas the erythrocytes and leukocytes are whole cells. Platelets play an important role in hemostasis and are responsible for forming the initial plug at sites of vascular injury (Akers R, Denbow D, 2008).

**Blood Plasma:**

The remaining element contributing to equine whole blood is the liquid portion, known as plasma. Equine plasma is uniquely orange or yellow, whereas most mammalian plasma is straw colored (Morris D, 2002). As much as 76% of the whole blood in a healthy adult horse may be plasma (Ziemer E, Bloom J, 1999). As discussed previously, greater than 90% of this nonliving liquid matrix is water (Akers R, Denbow
Despite its perceived simplicity, equine plasma contains nutrients, gases, hormones, minerals and proteins all of which perform innumerable functions responsible for the maintenance of life. Nutrients found in plasma are absorbed from the gastrointestinal tract or produced in the liver and include glucose, amino acids and lipids. Oxygen and carbon dioxide are transported in plasma along with calcium, chloride, potassium and other minerals. Proteins are the most abundant solute in equine plasma and have countless roles. The plasma proteins regulate biochemical reactions, act as carriers for other plasma constituents, provide colloid osmotic pressure to maintain a proper intravascular volume, participate in coagulation and function in immunity (Eckersall P, 2008).

**Plasma Proteins:**

The plasma protein concentration in healthy adult horses ranges from approximately 6.0-8.5g/dl (Duncan J, Prasse K, 1986). The majority of these plasma proteins are synthesized by and secreted from hepatocytes. Plasma proteins may be separated and identified based upon the process of protein electrophoresis (Figure 1.1). Protein electrophoresis separates proteins according to charge and size, yielding albumin and globulins (Eades S, 1999). Albumin is a discrete molecular compound identified by its sharp, narrow peak nearest the anode region on the electrophoretogram (Morris D, Johnston J, 2002). The globulins are a heterogeneous mixture of various protein types, which migrate in groups in the electric field as families of proteins identified as α, β and γ globulins. The α and β globulins are quite diverse and include acute phase proteins, complement proteins, lipoproteins, coagulation proteins and others. In contrast, immunoglobulins (Ig) predominate as the protein migrating into the γ globulin fraction.
(Eades S, 1999, Eckersall P, 2008). Unlike the other major plasma proteins, immunoglobulins are synthesized by B lymphocytes and not the liver (Eckersall P, 2008).

Healthy adult horses have 2.5-4.1g/dl albumin in their plasma, which makes it the most abundant single protein found in equine plasma. Albumin is synthesized in the cytoplasm of hepatocytes, with the synthetic rate controlled by colloid osmotic pressure and hormones (Eckersall P, 2008). About 30-40% of albumin is in blood, while the remainder is in interstitial spaces. The rate of turnover for albumin differs with species and is related to body size. The plasma half-life of equine albumin is 19.4 days in comparison to dogs and mice, which have albumin half-lives of 8.2 and 1.9 days, respectively. Albumin functions to maintain blood volume and oncotic pressure, accounting for 75-80% of colloid osmotic pressure (Eckersall P, 2008, Morris D, Johnston J, 2002). Albumin also binds and transports metabolites including fatty acids, cholesterol, metal ions and bilirubin. Similarly, administered pharmacological agents interact with albumin at four discrete binding sites and are transported throughout the

![Figure 1.1](image_url)  
**Figure 1.1** Electrophoretogram of agarose gel protein electrophoresis. Adapted from Tizard I, 2004.
circulatory system. Finally, albumin may be considered the predominate antioxidant in blood by acting to scavenge reactive oxygen and peroxynitrite radicals (Eckersall P, 2008).

Complement is a collection of interacting plasma proteins that participate in a cascade of events, which leads to the opsonization of foreign cells or particles. These proteins are zymogens and activate one another through a series of proteolytic cleavages (Eckersall P, 2008). Greater than 30 different complement proteins have been identified (Tizard I, 2004). The complement proteins migrate within the β globulin fraction during protein electrophoresis. It is possible to see a dramatic increase in complement concentrations in horses that experience inflammation (Eades S, 1999).

A significant amount of plasma protein is associated with lipid in the form of lipoproteins. Lipoproteins demonstrate both α and β globulin mobility on electrophoretograms, but it is more common to distinguish these proteins based on their density as determined by ultracentrifugation. The proteins are characterized as very low-density, low-density and high-density lipoproteins. These lipoproteins function to transport lipid between the liver and peripheral tissues. Chylomicrons are another class of lipoprotein and function to transport dietary lipid from the intestine (Eckersall P, 2008). The phenomenon of equine hyperlipidemia was reported in the 1960s and is the result of anorexia and increased lipolysis (Gainer J, Amster R, Needham J, et al., 1966, Hadlow W, 1962). It has been shown that fasting increases plasma levels of long chain fatty acids in horses. Presumably, the equine liver removes these long chain fatty acids, reesterifies them into triacylglycerol and releases them back into the plasma as very low-density lipoproteins (Bruss M, 2008). Therefore, it is important to rule out fasting as a
cause of increased lipoprotein concentrations when evaluating biochemical analytes of equine patients.

Acute phase proteins are a group of plasma proteins committed to defending hosts against pathological damage and restoring homeostasis. Acute phase protein production is an integral component of the innate immune system and generates the first reaction to pathogens and tissue injury (Abbas A, Lichtman A, 2005). The production of acute phase proteins is controlled by proinflammatory cytokines, which are released from sites of tissue damage. The liver is the major source for acute phase protein production, but mammary glands, lungs and intestine are also capable of significant synthesis. Plasma concentration of these proteins may increase by greater than 25% in response to inflammation, infection or trauma. As a result, these proteins are used as quantitative markers in general health screenings, diagnosis of disease, monitoring responses to therapy and as prognostic indicators. These particular plasma proteins migrate into both α and β globulin fractions during electrophoresis. The acute phase proteins include haptoglobin, serum amyloid A, protein C, ceruloplasmin, fibrinogen and others (Eckersall P, 2008).

Serum amyloid A and fibrinogen are particularly important acute phase proteins in horses. Serum amyloid A is characterized as a major acute phase protein in horses, with a large dynamic range between the resting concentration in healthy subjects and the concentration obtained from subjects with inflammation or infection (Eckersall P, 2008, Tizard I, 2004). Increased serum amyloid A concentrations have been reported in horses with enteritis, pneumonia, diarrhea, arthritis and post-surgery (Eckersall P, 2008). Similarly, fibrinogen is consistently increased during equine inflammation. In terms of
mass of protein, fibrinogen represents the greatest proportion of plasma protein synthesized during an acute phase response. Fibrinogen concentration is of unique value in the detection of newborn foals infected or exposed to inflammatory placental disease in utero. These foals may have fibrinogen levels greater than 1,000mg/dl at birth, when normal is 100-400mg/dl (Vaala W, House J, 2002). Fibrinogen is a relatively large plasma protein with \( \beta \) mobility on electrophoretograms (Eades S, 1999). However, serum rather than plasma is routinely used in the performance of protein electrophoresis (Eckersall P, 2008). Because serum is the liquid component of clotted blood, it lacks coagulation factors. Therefore, fibrinogen, which functions in coagulation, and the other clotting factors are usually not measured.

**Immunoglobulins:**

Immunoglobulins, also known as antibodies, are the major plasma protein with \( \gamma \) globulin mobility on protein electrophoresis (Eades S, 1999, Eckersall P, 2008). Immunoglobulin molecules exist as the membrane bound receptors of B lymphocytes and as secreted antibodies found in bodily fluids. All immunoglobulins share the same basic structural characteristics (Figure 1.2). Immunoglobulin molecules possess a symmetric core design composed of two identical light chains and two identical heavy chains. Each light chain is linked to one heavy chain via a disulfide bond and the two heavy chains are attached to one another via disulfide bonds. Both light and heavy chains contain homologous, repeating units of approximately 110 amino acid residues, which fold into a globular motif called the immunoglobulin domain. Each light chain is made up of one constant region Ig domain and one variable region Ig domain. The heavy chains are composed of 3-4 constant region Ig domains and one variable region Ig domain. The
variable regions are so named because they contain variable amino acid sequences that
distinguish antibodies produced by one clone of B lymphocytes from those produced by
another clone of B lymphocytes. The variable region of one heavy chain and the variable
region of one light chain are oriented next to one another to form the antigen-binding site.
More specifically, there are three short stretches of hypervariable segments in both the
light and heavy chain variable regions. These highly variable segments are each
approximately 10 amino acid residues in length and flanked by more conserved
sequences that make up the Ig domain of the variable region. The three hypervariable
segments in the light chain and the three hypervariable segments in the heavy chain are
arranged in a three-dimensional space to form the antigen-binding surface. The
hypervariable regions are also called complementarity-determining regions because these
sequences form a binding surface that is complementary to the three-dimensional
structure of antigen. Because the structural unit of every immunoglobulin molecule
contains two heavy chains and two light chains, it has two antigen-binding sites. The
constant region domains are separate from the variable region domains. They do not
function in antigen recognition, but rather the heavy chain constant regions mediate the
effector functions of the antibody. When in the membrane bound form, the carboxyl
terminal ends of the constant region heavy chains are anchored to the plasma membrane
of the B lymphocytes. The constant region of the light chains does not mediate effector
functions or attach to the B cell membranes. The exact function of constant region light
chains is unknown. There are five recognized classes of heavy chain constant regions (α,
δ, ε, γ and μ) and two recognized classes of light chain constant regions (κ and λ).
Immunoglobulin molecules are divided into five isotypes based on their class of heavy
The production of secreted immunoglobulins possessing different isotypes is a complex process that begins with naïve B lymphocytes. Mature antigen-responsive B lymphocytes develop in the bone marrow and then populate peripheral lymphoid organs. These naïve B cells home to follicles in the spleen, lymph nodes and mucosal lymphoid tissues to interact with foreign antigens. Each B cell is equipped with 200,000-500,000 identical, highly specific IgM and IgD bound antigen receptors (Tizard I, 2004). Antigen recognition by the immunoglobulin receptors initiates B cell activation and marks the beginning of the humoral immune response. B cell activation also requires a second
signal provided by the complement protein, C3d. The requirement of the second signal ensures that B lymphocyte responses most likely occur when complement activating antigens are encountered. Binding of specific antigen and C3d initiates a series of signaling cascades that lead to the functional activation of a B cell. As a result, an activated B lymphocyte mildly proliferates, demonstrates increased survival, increases cytokine receptor expression, up regulates expression of B7 molecules and begins to secrete IgM. The secreted form of immunoglobulin is identical to the bound receptor, except at the carboxyl terminus. A change in the processing of the heavy chain messenger RNA (mRNA) results in the production of a short tailpiece that cannot be anchored to the B cell membrane and the immunoglobulin molecule is secreted. The activated B cell then internalizes the antigen into vesicles via receptor-mediated endocytosis. Protein antigens are processed into peptides and presented on the B cell surface in association with class II major histocompatibility complex (MHC) molecules. Next, the activated B lymphocyte migrates to T cell-rich areas within the lymphoid tissue. Here, specific helper T lymphocytes recognize peptides displayed by the B lymphocyte and direct B lymphocyte proliferation, differentiation and class switching. The helper T lymphocytes stimulate B cell clonal expansion through production of cytokines interleukin (IL)-2, IL-4 and IL-5. Some of the progeny B cells resulting from the T cell-dependent proliferation differentiate into antibody-secreting effector cells. Many of these antibody-secreting cells become morphologically distinct plasma cells. The long-lived plasma cells migrate to the bone marrow where they continue to secrete abundant amounts of IgM. Other progeny of activated IgM and IgD-expressing B cells undergo heavy chain isotype switching. Isotype switching occurs in response to T cell
mediated CD40 engagement and cytokine production. The process of switch recombination allows for the production of isotypes IgA, IgE and IgG, each of which mediate different effector functions. Finally, B cells migrate to the middle of lymphoid follicles and form germinal centers. Here, B cells undergo rapid proliferation, affinity maturation and further differentiation. The doubling time of B cells located in germinal centers is estimated to be only 6-12 hours. As these cells divide, they acquire point mutations in their immunoglobulin variable genes at an extremely high rate. This somatic hypermutation in the variable regions alters the binding affinity of B cell receptors. Follicular dendritic cells (FDC) present in germinal centers display antigens and only the B cells binding with high affinity are selected to survive. This process of affinity maturation generates antibodies with increased capacity to bind antigens. Selected B cells exit the germinal centers and develop into high-affinity antibody secretors. Other selected B cells differentiate into quiescent, long-lived memory cells. These B lymphocytes typically possess high-affinity, isotype switched immunoglobulin receptors. Upon secondary exposure to an antigen, these cells are responsible for increased quality, quantity and timing of the humoral immune response. Instead of a slow and relatively small increase in circulating IgM as seen after initial antigen exposure, there is a rapid and large increase in production of high-affinity, isotype switched antibodies (Abbas A, Lichtman A, 2005, Tizard I, 2004).

As discussed, there are five different immunoglobulin isotypes as determined by the class of heavy chain constant region incorporated into the immunoglobulin molecule. Naïve B cells only express membrane bound IgD and IgM. During switch recombination, activated B cells gain the ability to produce IgA, IgE or IgG. Following
the process of cellular differentiation, selected B cells become capable of immunoglobulin secretion. Effector function and distribution of secreted immunoglobulins are dictated by their isotype. Every immunoglobulin molecule also has either two identical κ or λ light chains. However, light chains do not influence the effector function or distribution of secreted immunoglobulins (Abbas A, Lichtman A, 2005, Tizard I, 2004).

Immunoglobulin D and IgM are simultaneously expressed as membrane bound receptors on naïve B lymphocytes. Immunoglobulin D’s major role is to function as a B cell receptor. Only very small amounts of IgD are secreted into blood with subsequent identification in the plasma. In contrast, significant concentrations of IgM are identified in plasma. The secreted form of IgM exists as a pentamer, which consists of five identical IgM molecules linked together by disulfide bonds (Figure 1.3). Immunoglobulin M is the major immunoglobulin produced during a primary immune response and functions in complement activation, opsonization, agglutination and neutralization of antigen (Abbas A, Lichtman A, 2005, Tizard I, 2004).

During switch recombination, IgA is generated in response to the cytokines IL-5 and transforming growth factor (TGF)-β. B cells located deep to the body’s epithelial surfaces secrete IgA as a dimer (Figure 1.3). Immunoglobulin A is efficiently transported through epithelia and into mucosal secretions, where it is the primary immunoglobulin that participates in mucosal immunity. As such, it protects the respiratory, intestinal and urogenital tracts, the mammary glands and eyes from microbial invasion. Specifically, IgA functions to agglutinate particulate antigens, neutralize viruses and prevent antigen binding to specific receptors (Abbas A, Lichtman A, 2005, Tizard I, 2004).
Immunoglobulin E is found in very low concentrations in plasma and has a relatively short half-life of 2-3 days (Tizard I, 2004). Secreted as a monomer, this immunoglobulin is responsible for the destruction of helminthic parasites and immediate or type I hypersensitivity reactions (Figure 1.3). Helminths activate the TH2 subset of helper T lymphocytes, which produce IL-4. Interleukin-4 induces B cell switching to the IgE isotype, which participates in eosinophil-mediated killing of helminths. Multiple IgE antibodies bind to the surface of the parasites. Eosinophils bind to the Fc region of IgE antibodies and become activated. The activated eosinophils release their granule content, which kills the helminths. Exposure to other antigens may result in activation of TH2 lymphocytes. Specific antigens, known as allergens, activate these T cells and stimulate production of IgE antibodies, which bind to Fc receptors of mast cells and basophils. Re-exposure to the allergen results in activation of the mast cells and basophils with release of their contents, including vasoactive amines, lipid mediators and cytokines. Increased vascular permeability, smooth muscle contraction, tissue damage, vasodilation and bronchoconstriction may result. This reaction is known as immediate hypersensitivity because it begins quickly upon antigen re-exposure and has major pathological consequences (Abbas A, Lichtman A, 2005, Tizard I, 2004).

The most abundant antibody in the plasma is IgG. In contrast to IgE, IgG has a relatively long half-life of about 3 weeks (Abbas A, Lichtman A, 2005). Immunoglobulin G is secreted as a monomer from B cells in the spleen, lymph nodes and bone marrow (Figure 1.3). During switch recombination, TH1 lymphocytes promote the formation of IgG through production of interferon (IFN)-γ and other cytokines. Immunoglobulin G protects the host through numerous effector functions.
Immunoglobulin G antibodies inhibit or neutralize microbes by binding to their surface and interfering with their ability to enter host cells. Similarly, IgG antibodies sterically hinder the interaction of toxins with host cells, which prevents tissue damage and disease. Immunoglobulin G antibodies also promote the removal of antigens through phagocytosis. The IgG antibodies coat or opsonize invading microbes. Macrophages or neutrophils bind to the IgG via their Fc receptor and engulf the bound microbes. Immunoglobulin G is also capable of activating the classical pathway of complement. Other IgG antibodies bind to the surface of infected cells. The Fc region of these IgG antibodies is recognized by natural killer cells, which destroy the opsonized cell. Maternally produced IgG plays a critical role in protecting neonatal mammals from infection. Neonates lack the ability to mount effective immune responses against pathogens during the first several months of life. Therefore, they rely on passive immunity provided by maternal antibodies. In some mammals a protective concentration of IgG is transported across the placenta, while in other mammals protective concentrations of IgG are ingested and absorbed from colostrum (Abbas A, Lichtman A, 2005, Tizard I, 2004).

Figure 1.3 Plasma cells secreting immunoglobulin molecules. A. IgM secreted as a pentamer. B. IgA secreted as a dimer. C. IgE or IgG secreted as a monomer. Permission granted to use illustration published in Cellular and Molecular Immunology 5th edition, Abbas A and Lichtman A, Copyright Elsevier 2005.
The concept of five different antibody isotypes performing separate effector functions is generally conserved across mammalian species (Wagner B, Miller D, Lear T, et al., 2004). However, considerable variation always exists. For example, the immunoglobulin heavy chain constant region δ gene has not been identified in rabbits and several other animals (Tizard I, 2004, Wagner B, Miller D, Lear T, et al., 2004). Therefore, these species do not produce IgD. Furthermore, the IgD proteins detected in human beings, cows, pigs and sheep contain three constant region heavy chain immunoglobulin domains, whereas the IgD detected in mice contain only two constant region heavy chain immunoglobulin domains (Tizard I, 2004). Variation also exists in the number of subclasses expressed for each antibody isotype. Rabbits express thirteen different subclasses of IgA. In contrast, human beings produce two IgA antibodies and most other mammals produce only one IgA antibody (Wagner B, Miller D, Lear T, et al., 2004). Variation is also observed in the ratio of κ to λ light chains incorporated into the immunoglobulin molecules. In human beings, approximately 60% of the antibodies have κ light chains and the other 40% have λ light chains. In contrast, the κ to λ light chain ratio in mice is approximately 10 to 1 (Abbas A, Lichtman A, 2005). Even more extraordinary, members of the camel family regularly produce two subclasses of IgG that have no light chains. Despite lacking light chains, these IgG antibodies are functionally sound (Tizard I, 2004, Wagner B, Miller D, Lear T, et al., 2004).

**Equine Immunoglobulins:**

As expected, the antibodies produced by horses demonstrate their own unique features. Until recently, it was believed that horses produce only IgA, IgE, IgG and IgM. However, the sequence for the equine immunoglobulin heavy chain constant region δ
gene was identified in 2004 (Wagner B, Miller D, Lear T, et al., 2004). In this study, expression of transmembrane and secreted forms of the horse immunoglobulin δ gene was confirmed at the mRNA level, suggesting that IgD may be expressed by equine B cells. The ratio of κ to λ light chains is also interesting in horses. Over 90% of equine antibodies contain λ light chains, corresponding to a 1:13 ratio (Wagner B, 2006). Finally, horses express only one IgA, IgD, IgE and IgM antibody. However, they express seven different subclasses of IgG, more than any other mammal studied thus far (Wagner B, Miller D, Lear T, et al., 2004).

Through the early 2000s, it was believed that horses generate five subclasses of IgG. The nomenclature for these antibodies was based on antigenic differences, serological properties and electrophoretic behavior (Wagner B, 2006). Equine IgG subclasses included IgGa, IgGb, IgGc, IgG(T) and IgG(B) (the aggregating immunoglobulin). However, recent mapping of the equine immunoglobulin heavy chain constant region revealed seven different γ genes, corresponding to seven different subclasses of IgG now designated IgG1-IgG7 based on their respective gene (Wagner B, Miller D, Lear T, et al., 2004). Today, most of the earlier identified IgG subclasses have been linked to at least one of the seven γ genes (Lewis M, Wagner B, Woof J, 2008, Wagner B, 2006). Unfortunately, the literature defining these new subclasses of IgG is unclear, inconsistent and incomplete (Table 1.1). Results of studies published in 2006 and 2008 reported conflicting information regarding the nomenclature reassignment (Lewis M, Wagner B, Woof J, 2008, Wagner B, 2006). Also, neither of the investigations addressed the reassignment of equine IgG(B).
<table>
<thead>
<tr>
<th>Original Nomenclature</th>
<th>2006 Designation(^1)</th>
<th>2008 Designation(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgGa</td>
<td>IgG1, IgG2</td>
<td>IgG1</td>
</tr>
<tr>
<td>IgGb</td>
<td>IgG4</td>
<td>IgG4, IgG7</td>
</tr>
<tr>
<td>IgGc</td>
<td>IgG6, IgG7</td>
<td>IgG6</td>
</tr>
<tr>
<td>IgG(T)</td>
<td>IgG3, IgG5</td>
<td>IgG3, IgG5</td>
</tr>
<tr>
<td>IgG(B)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

\(^1\) (Wagner B, 2006)  
\(^2\) (Lewis M, Wagner B, Woof J, 2008)  
n/a = not applicable

The distribution of secreted immunoglobulins in bodily fluids of healthy adult horses is relatively straightforward. Immunoglobulin G is the major antibody in equine plasma and is believed to be dominated by IgG4 (Tizard I, 2004, Wagner B, 2006). Both IgA and IgG are present in mucosal secretions. Findings from an examination of equine tears determined IgA to be the predominating antibody (Wagner B, 2006). However, significant concentrations of both IgA and IgG have been detected in horses’ urogenital and respiratory tracts (Tizard I, 2004). Finally, results of a study on equine colostrum revealed highly increased concentrations of IgG1, IgG3, IgG4, IgG5 and IgA, with the predominating antibody in colostrum believed to be IgG4 (Wagner B, 2006).

**Measurement of Equine IgG:**

The integrity of equine B lymphocytes and their ability to produce antibodies, especially IgG, is of critical importance. Measuring the total IgG concentration in the plasma or serum of adult horses provides valuable information regarding their humoral immune system and their ability to prevent disease (Eades S, 1999). It has been long established that healthy adult horses have a total IgG concentration of 1,000-1,500mg/dl (Tizard I, 2004). However, few sound studies evaluated the equine humoral response and quantified their immunoglobulin concentrations (Lunn D, Horohov D, 2004). Findings
from a recent publication from Brazil suggest that total IgG concentration for adult horses is actually 2,704 +/- 1,424mg/dl (de Carmargo M, Kuribayashi J, Bombardieri C, et al., 2008). This reference interval is strikingly higher and has more variation in comparison to previously described values. Two other separate studies reported similar findings in regard to total IgG concentrations in adult horses (Kohn C, Knight D, Hueston W, et al., 1989, Sheoran A, Timoney J, Holmes M, et al., 2000).

Measurement of total IgG concentration in neonatal foals is of great value in predicting their immune status. Mares have diffuse epitheliochorial placentation, which prevents the transplacental transfer of maternal antibodies in utero (Erhard M, Luft C, Remler H, et al., 2001, McCue P, 2007). Consequently, foals depend on the ingestion and absorption of immunoglobulin-rich colostrum to provide passive immune protection. Failure of passive transfer (FPT) of maternal antibodies puts foals at considerable risk for development of infectious diseases (McCue P, 2007). Therefore, knowledge of the neonates’ IgG concentration greatly assists in detection of FPT, which allows for intervention to reduce morbidity and mortality (Metzger N, Hinchcliff K, Hardy J, et al., 2006). It is generally accepted that a plasma or serum IgG concentration of >800mg/dl 13-16 hours postnatum indicates the successful transfer of passive immunity (Erhard M, Luft C, Remler H, et al., 2001). Foals with IgG concentrations <200mg/dl are considered to have complete FPT and will likely require medical attention. Partial FPT has been described as IgG concentrations between 200-400mg/dl and 200-800mg/dl (McCue P, 2007, Metzger N, Hinchcliff K, Hardy J, et al., 2006). Many clinicians strictly use 800mg/dl IgG as the cutoff for defining successful passive transfer. However, other veterinarians consider foals with IgG concentrations >400mg/dl but <800mg/dl to be
protected. Because many factors influence the susceptibility of foals to disease, it is not possible to determine one IgG concentration that is ideal for all situations (Metzger, N, Hinchcliff, K, Hardy, J, et al., 2006).

Different assays and methods have been used over the years to measure IgG concentration in equine patients. Radial immunodiffusion (RID) and enzyme-linked immunosorbant assay (ELISA) have been used to yield quantitative results. Other methods provide semi-quantitative information, such as glutaraldehyde coagulation and zinc sulfate turbidity tests (Beetson S, Hilbert B, Mills J, 1985, McCue P, 2007). In contrast, measuring patients’ total protein via refractometry provides only a crude estimate of their IgG concentration (Davis R, Giguere S, 2005). The different techniques are employed based upon availability, cost, timing and other factors.

Radial immunodiffusion has been universally accepted as the gold standard for measurement of IgG concentration in horses (Lunn D, Horohov D, 2004). The test is based on the diffusion of IgG from a circular well into a homogenous gel containing specific antiserum. A ring of precipitation forms where the IgG and its antibody combine (Figure 1.4). The ring continues to grow until equilibrium is reached. The diameter of the precipitation ring is directly related to the amount of IgG in the well. Solutions with known concentrations of IgG are used to construct a standard curve, against which sample solutions are compared (Tizard, I, 2004). Sample solutions used in performing RID are typically serum or plasma. Although RID is considered the gold standard in determination of IgG concentration, it does have drawbacks. One major disadvantage is the long incubation time (18-24 hours) required to obtain results (Lunn D, Horohov D, 2004, McCue P, 2007). Therefore, the assay is not very practical in detection of FPT,
where rapid results are necessary to direct therapeutic action. Also, few studies have investigated the agreement of results among commercially available RID assays (Davis R, Giguere S, 2005). In addition, some manufacturers of equine RID kits market tests for measurement of total IgG and others for measurement of subclasses of IgG. Kits designed to measure subclasses are still marketed under the original IgG nomenclature. This makes it difficult for clinicians and researchers to report results using the currently accepted nomenclature, which causes more confusion. Manufacturers of the kits claim that the precipitation ring is sharp and easy to read, but the author of this publication does not find this to be entirely true. On occasion, the generated rings are blurry, displayed in duplicate or are difficult to measure against the graduated scale on the plate.

Equine IgG concentrations may also be determined using ELISA. Numerous variations of ELISA exist, however they are all based on similar principles. One example is the antibody sandwich ELISA (Figure 1.5). In this variation, microwells of a plate are coated with a specific capture antibody, which recognizes equine IgG. Samples of horse serum or plasma are added to the wells and any IgG in the samples is captured by the antibody. Following incubation and washing, a detector antibody conjugated with an enzyme is added to the wells. The detector antibody remains in the wells following
washing only if bound to captured IgG. Lastly, a substrate is added with resultant color change only if the detector antibody conjugated with the enzyme is present. Therefore, the intensity of the color that develops is proportional to the amount of enzyme-linked antibody that is bound, which is proportional to the amount of IgG present in the sample (Figure 1.6). Optical density of samples and known standards are calculated using a plate reader. Concentration of IgG in each sample is then determined from the standard curve (Tizard I, 2004). Enzyme-linked immunosorbent assays have become very popular for quantifying equine IgG levels. A recent study published in the American Journal of Veterinary Research used inhibition ELISA rather than RID to quantify immunoglobulin isotypes in horses (Sheoran A, Timoney J, Holmes M, et al., 2000). Compared to RID, ELISA is much faster, which makes it a better assay for detecting FPT. A semi-quantitative, stall-side ELISA has been developed especially for this purpose (Idexx SNAP Foal IgG Test Kit, Greensboro, NC). Similar to RID, ELISAs may be designed to measure total equine IgG or specific subclasses of IgG. Unfortunately, the currently available anti-horse IgG capture antibodies are defined under the original nomenclature. This issue does not influence ELISAs that measure total IgG, but ELISAs that measure subclasses of equine IgG are affected. A recent study evaluated ELISAs that use polyclonal anti-horse antibodies directed against IgGa, IgGb, IgGc and IgG(T) (Lewis M, Wagner B, Woof J, 2008). The investigators found that polyclonal antibody directed against horse IgGa recognized only IgG1. In contrast, goat anti-horse IgGb demonstrated reactivity with IgG1, IgG4 and IgG7. The anti-horse IgGc antibody recognized IgG6. Goat anti-horse IgG(T) polyclonal antibody reacted most strongly with IgG5, but also
recognized IgG2 and IgG3. The results of this investigation highlight the need for capture antibodies directed against the currently recognized IgG subclasses.

**Figure 1.5** Antibody sandwich ELISA. Adapted from Lunn D, Horohov D, 2004.

**Figure 1.6** ELISA. Microwells of the plate demonstrate different intensities of color development proportional to the amount of IgG present in each well.
A number of semi-quantitative tests that measure equine IgG have been employed over the years. Many of these assays are relatively rapid, which make them ideal for detection of FPT (Davis R, Giguere S, 2005). One example of such a test is the glutaraldehyde coagulation test. A blood sample is obtained from a foal and its serum is added to 10% and 20% glutaraldehyde solutions. Time is observed for gel formation in samples. Serum samples containing ≥800mg/dl IgG produce a solid gel in the 10% solution within ten minutes. Foals with IgG concentrations between 400 and 800mg/dl demonstrate coagulation in the 10% solution within one hour. Foals with IgG concentrations between 200 and 400mg/dl demonstrate coagulation in the 20% solution within one hour. Foals with complete FPT fail to produce a solid gel in the 20% solution after one hour. Along with being rapid, the test is relatively sensitive and specific, simple and inexpensive (Beetson S, Hilbert B, Mills J, 1985).

**Hypoproteinemia:**

In addition to FPT, a number of diseases and conditions may reduce significantly the concentration of plasma proteins in equine patients. A hypoproteinemic state may result owing to either decreased protein production or increased protein loss. Peritonitis, gastrointestinal ulceration and forms of renal disease cause hypoproteinemia through increased loss of plasma proteins. Granulomatous enteritis, gastrointestinal neoplasia, intestinal parasitism and nonsteroidal anti-inflammatory drug (NSAID) toxicity result in protein-losing enteropathies, which also decrease the total plasma protein concentration. Immunodeficiency and liver disease may reduce plasma protein concentrations due to decreased production. Other dysproteinemias in association with equine liver diseases are common. Clinically, hypoproteinemic horses may have edema of the ventral midline,
limbs, submandibular region and lungs. During thoracic auscultation, crepitations may be detected. Horses with a significant decrease in $\gamma$ globulin concentrations, as a result of immunodeficiency, also may have recurrent infections, septicemia or pneumonia (Eades S, 1999).

Instead of a decrease in total plasma protein concentration, other horses may experience a marked decrease or dysfunction in just one very specific plasma protein. Hemophilia A and von Willebrand’s disease are inherited bleeding disorders that result from deficiencies in a single coagulation factor. Hemophilia A has been reported in Thoroughbreds, Standardbreds, Quarter Horses and Arabians (Zimmel D, 2003). It is transmitted as an x-linked recessive trait and consequently only affects males. Affected horses present with severe spontaneous hemorrhage early in life owing to deficiency in coagulation factor VIII. Because of the severity of the disease, most horses die or are euthanized between 6 months to 3 years of age. Von Willebrand’s disease is a rare disorder reported in both sexes of Thoroughbreds and Quarter Horses. This inherited bleeding disorder results from a quantitative or qualitative defect in von Willebrand’s factor (vWF). This defect interferes with formation of a platelet plug, which results in mild hemorrhagic diathesis. Clinical signs include recurrent epistaxis and surface bleeding following surgery or minor trauma. Unlike hemophilia A, von Willebrand’s disease may not be diagnosed until later in life (Johnstone I, 1999, Zimmel D, 2003).

**Plasma Therapy:**

Primary therapy for treatment of renal disease, peritonitis, FPT and parasitism may have very little in common. Long-term, curative therapy for some of the discussed conditions, like hemophilia A, does not exist (Johnstone I, 1999, Zimmel D, 2003).
However, horses with any of the described conditions would benefit from plasma transfusion. Through its ability to provide transport proteins, clotting factors, immunoglobulins and enzymes, along with its role in restoration of oncotic pressure, plasma administration is a valuable primary or supportive treatment for numerous equine diseases (Morris D, 1999, Porter M, Green E, 2003).

Plasma therapy offers several advantages over whole blood transfusion in the treatment of horses with these conditions. Plasma infusions provide recipients with specific components they are lacking. Whereas whole blood transfusions limit the maximal volume of plasma delivered owing to the inappropriate simultaneous supplementation with red blood cells (Porter M, Green E, 2003). Provision of plasma through whole blood transfusion could result in erythrocytosis and hyperviscosity. Also, infusion with only plasma avoids exposure to foreign erythrocyte antigens and hemolytic reactions (Mischke R, 2005). Plasma is easily stored frozen at -20°C for several years, with most plasma proteins surviving at least one freeze-thaw cycle before administration. In contrast, it is recommended that whole blood be used immediately or within several days if refrigerated at 4°C (Morris D, 1999). Although whole blood transfusions are more common in veterinary medicine, blood products such as fresh or fresh frozen plasma are usually the therapy of choice. The only true indication for whole blood transfusion is in the event of acute massive hemorrhage, which is when equine patients would experience a dramatic drop in both red blood cells and plasma volume (Mischke R, 2005, Porter M, Green E, 2003).
Equine Plasma Market:

Horse plasma has other valuable uses beyond transfusion into protein deficient equidae. Horse plasma and its constituents are harvested for use in laboratory settings. It is sold commercially for incorporation into various culture media used by diagnostic laboratories, may serve as the matrix in pharmacokinetic studies that determine the half-lives of therapeutic compounds and polyclonal antibodies generated in equine subjects are harvested from their plasma and used in scientific assays.

Very specific equine plasma, hyperimmune equine plasma, occupies an important niche in the veterinary market. This IgG-rich plasma is commercially manufactured and sold for the prevention or treatment of specific equine infectious diseases (Brook D, 1989, Magdesian K, Brook D, Wickler S, 1992, Porter M, Green E, 2003). These products are generated by repeatedly inoculating donor horses against a specific infectious agent (Porter M, Green E, 2003). As a result, the donor horses produce large quantities of circulating high-affinity antibodies. Plasma containing the IgG antibodies is harvested from the donors and sold as hyperimmune plasma for transfusion. Currently available products include Rhodococcus equi antibody hyperimmune plasma, Clostridium botulinum type B antitoxin hyperimmune plasma, West Nile Virus antibody hyperimmune plasma and others (Plasvacc USA, Templeton, CA). These equine plasma products are classified as biological products and are therefore under the governing authority of the United States Department of Agriculture (USDA) (Brook D, 1989). Title 9 Code of Federal Regulations is the regulatory document that outlines all requirements for biological products licensed by the USDA (http://www.access.gpo.gov/nara/cfr/waisidx_03/9cfrv1_03.html). The document
specifies that all animals used for antibody products shall be healthy, based upon physical
examination and tests for infectious diseases. Prior to use of horses for antibody
production, all donors must be tested and found negative for equine infectious anemia
(EIA), piroplasmosis (Babesia equi and Babesia caballi), dourine (Trypanosoma
equiperdum), glanders (Burkholderia mallei) and brucellosis (Brucella abortus). Once
accepted as donors, the animals are tested for infectious diseases at regular intervals. The
donor herds are maintained at the licensed establishments and kept separate from other
animals. The harvested antibody products must be safe, efficacious and disease-free.
The hyperimmune equine plasma has to meet potency standards and demonstrate a
defined shelf life. The final USDA licensed products require accurate labeling and
appropriate storage and transportation (Brook D, 1989,
http://www.access.gpo.gov/nara/cfr/waisidx_03/9cfrv1_03.html).

Equine plasma also plays an important role in the human pharmaceutical industry.
Similar to the process of creating hyperimmune plasma for veterinary use, selected horses
are hyperimmunized against venoms, toxins and viruses clinically significant in the
human population. After obtaining the hyperimmune equine plasma, a series of
refinement steps are performed to produce antivenoms, antitoxins and similar antibody
agents for use in people. Traditionally, equine IgG was purified by sulfate-induced
precipitation or by caprylic acid-based fractionation, with resultant production of a
hyperimmune equine IgG product (Fernandes A, Kaundinya J, Daftary G, et al., 2008,
Wang L, Sun X, Ghosh R, 2008). However, the process has evolved over the last
century. Today, IgG is isolated from horse plasma by use of a precipitation or
fractionation technique and then digested with pepsin or papain. This enzymatic reaction
results in the production of antibody fragments F(ab)2 or Fab, respectively (Abbas A, Lichtman A, 2005) (Figure 1.7). The F(ab)2 and Fab antibody fragments are preferred over the whole IgG antibodies owing to their wide volume of distribution, ability to reach tissue compartments at a faster rate and decreased immunogenicity (Fernandes A, Kaundinya J, Daftary G, et al., 2008, Morais V, Massaldi H, 2005). When comparing F(ab)2 and Fab antibody fragments, the Fab fragments are less immunogenic and pharmacokinetically superior (Covell D, Barbet J, Holton O, et al., 1986, Mayers C, Veall S, Bedford R, et al., 2003). Despite the digestion process, fragments retain their activity and are biologically functional (Morais V, Massaldi H, 2005). Following enzymatic digestion, advanced purification techniques are used to obtain IgG fragments. Current purification techniques include ion-exchange chromatography, protein A or protein G based affinity chromatography, hydrophobic interaction chromatography and membrane based enhanced hybrid bioseparation (Fernandes A, Kaundinya J, Daftary G, et al., 2008, Wang L, Sun X, Ghosh R, 2008). The resultant highly-purified, hyperimmune equine F(ab)2 or Fab products are less likely to induce serum sickness, as seen with hyperimmune products used in the past (Abbas A, Lichtman A, 2005). Following treatment with the traditional products, patients would develop antibodies directed against animal proteins present in the hyperimmune products. Immune complexes would form and result in a type III or immune complex mediated hypersensitivity reaction. Clinical presentation of serum sickness includes rashes, arthralgia, fever, edema, lymphadenopathy and hypotension. The occurrence of serum sickness as a result of treatment with an equine-derived antibody product was first described by Dr. Clemens von Pirquet in 1911 (Abbas A, Lichtman A, 2005). At the time, patients with diphtheria
infections were administered serum from horses immunized against diphtheria toxin, which provided passive immunization in recipients. Today, equine derived diphtheria antitoxin and all other equine derived hyperimmune products produced in the United States are licensed for infectious disease prophylaxis and passive immunization only through approval by the Food and Drug Administration (FDA) (http://www.fda.gov/). These hyperimmune products are produced in accordance with current good manufacturing practices (cGMPs), which are regulations enforced by the FDA. Current good manufacturing practices require that manufacturing processes are clearly defined, controlled and validated. Records must be generated to document processes and systems must be available for recalling products. While the FDA governs the products produced in the United States, most equine derived hyperimmune agents that target spider, scorpion and snake venoms are produced overseas and are not licensed by the FDA.
Equine Plasma Collection:

Equine plasma has been recognized as a valuable resource for greater than a century. Over the last one hundred plus years, horse plasma has been harvested by use of several different techniques. The oldest method for collection of equine plasma is also the simplest. Early investigators merely collected whole blood and then relied on the rapid sedimentation rate of equine blood to obtain plasma (Feige K, Ehrat F, Kastner S, et al., 2003, Levine L, Broderick E, 1970, Morris D, 1999). The materials required to harvest plasma by this technique are rather minimal and the method is relatively uncomplicated. The investigators aseptically placed an intravenous catheter or large bore stainless steel needle into the jugular vein of donor horses. A tubing set extending from

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**Figure 1.7** Digestion of an IgG molecule with papain results in two Fab fragments and a single Fc fragment. Digestion of an IgG molecule with pepsin results in a single F(ab)2 fragment and multiple peptide fragments. Permission granted to use illustration published in Cellular and Molecular Immunology 5th edition, Abbas A and Lichtman A, Copyright Elsevier 2005.
the catheter or needle carried the whole blood into a glass collection container (Porter M, Green E, 2003). Prior to use, anticoagulant was added to the 8-9L collection jar and then the glass jar was autoclaved. Approximately 10-20% of the total blood volume was removed from each donor horse, with resultant anticoagulant to whole blood ratio of 1:10 (Green E, Ward G, 1974, Porter M, Green E, 2003). The jar and its contents were refrigerated for 12-24 hours, which allowed for sedimentation of cellular components. Plasma, which settled on top of the cells, was removed from the collection jar via a siphon or other mechanism and transported into a separate glass container. Following removal of plasma, the remaining cellular contents were discarded. This procedure was, and still is, performed safely at 30 day intervals (Green E, Ward G, 1974, Morris D, 1999, Porter M, Green E, 2003). Donor horses weighing 500kg can donate 4-8L of whole blood every 30 days, which yields approximately 2.5-5L of plasma monthly. The collection method is simple and inexpensive. However, the open collection system allows for possible bacterial contamination (Morris D, 1999). In addition, the collected plasma is not completely free of cellular elements. In a study evaluating plasma collection methods, researchers found that plasma harvested via gravity sedimentation contained a median of 935 erythrocytes and 2,900 leukocytes per µl (Feige K, Ehrat F, Kastner S, et al., 2003). The activity of factor VIII was also reduced significantly in plasma obtained by this collection method. The procedure is relatively lengthy, requiring time for whole blood collection, sedimentation and plasma removal.

**Equine Plasmapheresis:**

Two separate reports of manual equine plasmapheresis were published in the 1970s (Levine L, Broderick E, 1970, Green E, Ward G, 1974). These articles described a
new technique for equine plasma collection, which produced greater yields over time. The investigators of both groups collected equine whole blood, allowed for gravity sedimentation and removed the plasma, just as teams of investigators did in the past. However, with plasmapheresis the remaining cellular elements were not discarded following plasma extraction. Instead, concentrated cells were resuspended in a volume of isotonic saline similar to the volume of plasma removed. The resuspended blood cells were warmed in an incubator to 37°C and transfused back into respective donor horses.

Both groups performed manual plasmapheresis weekly, removing 10-20% of donors’ total blood volume during each procedure. Green and Ward plasmapheresed four 500-545kg adult horses for 35 consecutive weeks and reported no adverse effects. The donors’ PCV, protein levels and electrolyte values remained within normal intervals over the length of their investigation (Green E, Ward G, 1974). Levine and Broderick reported the performance of manual equine plasmapheresis with the Massachusetts Department of Public Health for greater than 20 years. These investigators claimed that weekly plasmapheresis had no apparent adverse effect on the condition of the donors or on the antibody titers of their plasma (Levine L, Broderick E, 1970).

Manual equine plasmapheresis demonstrates advantages over previously used plasma collection methods. Plasmapheresis restores blood cells to the donors, which allows for weekly collections instead of monthly collections. Moving from a traditional plasma collection technique to plasmapheresis is equivalent to increasing a production facility’s herd threefold. This makes plasmapheresis very valuable as demand for plasma increases and in countries where horses are in short supply or are expensive to maintain (Levine L, Broderick E, 1970). However, the process of manual plasmapheresis does
possess some drawbacks. Weekly procedures translate to weekly jugular
catheterizations. Damage to the jugular veins caused by the frequent introduction of
needles or catheters could delay or prevent manual plasmapheresis (Phillips A, Courtenay
J, Ruston R, et al., 1974). Again, the procedure is an open system, which allows for
possible bacterial contamination (Morris D, 1999). The process is also quite lengthy,
with even more time required for blood cell resuspension and transfusion. To reduce the
time associated with performance of manual plasmapheresis, other investigators began to
harvest the plasma in blood bags, which were centrifuged to achieve faster separation.
Researchers found that blood bag centrifugation not only reduced the time for plasma
separation, but also reduced the number of blood cells remaining in the product (Feige K,
contained a median of 615 erythrocytes and only 10 leukocytes per µl, significantly fewer
cells than plasma collected via gravity sedimentation.

Introduction of automated in-line blood cell separators in the early 1980s
revolutionized the plasmapheresis procedure (Bartges J, 1997, Klages C, 2006). These
instruments connect to donors’ catheters via plasmapheresis tubing sets, which include
the donors in a closed loop plasma collection system (Feige K, Ehrat F, Kastner S, et al.,
2003, Morris D, 1999). Instruments are equipped with pumps that remove whole blood
from donors and infuse anticoagulant at a controlled rate to the blood. The plasma is then
separated from the cellular elements by a variable speed centrifuge or membrane
filtration techniques, where plasma is diverted to an attached collection bag or collection
bottle, while cellular elements are returned to the donors (Bartges J, 1997, Feige K, Ehrat
provide fluid replacement during plasma collection and others provide fluid replacement after plasma collection is complete. Developed for use in the human healthcare industry, these instruments have been manipulated for the automated plasmapheresis of horses in commercial antibody enterprises and in the research arena (Feige K, Ehrat F, Kastner S, et al., 2003, Feige K, Ehrat F, Kastner S, et al., 2005, Klages C, 2006, Magdesian K, Brook D, Wickler S, 1992).

Automated equine plasmapheresis offers numerous advantages over more conventional plasma collection techniques. Automated plasmapheresis is performed in a closed sterile system, which greatly reduces the risk of bacterial contamination (Feige K, Ehrat F, Kastner S, et al., 2003, Morris D, 1999). A significantly greater amount of plasma is harvested in a significantly shorter period of time, which makes automated plasmapheresis a more efficient collection method (Feige K, Ehrat F, Kastner S, et al., 2003, Klages C, 2006). Automated equine plasmapheresis also minimizes the extracorporeal deficit by simultaneously harvesting plasma and returning blood cells (Bartges J, 1997). With automated plasmapheresis, the blood is processed through a sterile disposable tubing set and separation device. Therefore, the equipment is not used on multiple donors, the risk of cross-contamination is eliminated and there is no need for materials to be autoclaved prior to use (Klages C, 2006). Results from a study that evaluated equine plasma collected via automated centrifugation found almost complete removal of erythrocytes and leukocytes (Feige K, Ehrat F, Kastner S, et al., 2003). Activity of factor VIII was significantly greater in plasma produced by automated plasmapheresis in comparison to plasma harvested by gravity sedimentation. These advantages of automated equine plasmapheresis must be weighed against the cost
associated with the automated technique (Mischke R, 2005). The machines used to perform automated plasmapheresis are priced as high as $35,000 each. Cost of disposable tubing sets, training for individuals to operate the instruments and service to maintain or repair the devices must be considered. Moreover, the instruments, supporting supplies and operating manuals are designed for use with human subjects and as a result numerous logistical obstacles must be overcome before using the machines to harvest plasma from equine donors (Klages C, 2006).

While a number of facilities take advantage of automated plasmapheresis to harvest a large volume of sterile equine plasma, studies that examine automated equine plasmapheresis and the effect it has on both the donors and the product are limited. Findings from a study on the temporal effects of automated plasmapheresis on serum proteins in horses was published in the American Journal of Veterinary Research (Magdesian K, Brook D, Wickler S, 1992). The treatment group consisted of one mare and seven geldings of various breeds ranging in age from 5 to 21 years. All eight horses belonged to a commercial plasmapheresis program and had previously undergone automated plasmapheresis a mean of once every 30 days for 6 to 18 episodes without complication. Timing of plasmapheresis was based on each horse reaching a minimum total serum protein content of 6g/dl as determined by refractometry. For the investigation, the treated horses were subjected to approximately three hours of automated plasmapheresis by use of a Haemonetics instrument (Haemonetics Corp, Braintree, MA). During the procedure, lactated Ringer’s solution was infused into the donors along with the return of their blood cells. The procedure stopped when the protein concentration in the excurrent plasma decreased to approximately 5.5g/dl as determined
by refractometry. This resulted in collection of 4-11L of plasma per horse. The
investigators measured total protein, albumin and IgG concentrations prior to treatment,
immediately following treatment and over a 30-day period post-treatment. Total protein
was determined by the Biuret method, albumin was measured by spectrophotometry and
IgG concentrations were measured by RID (VMRD Inc, Pullman WA). Mean total
serum protein concentration in the eight treatment horses was 7.38g/dl +/- 0.53g/dl prior
to automated plasmapheresis. This baseline total protein value for the treatment horses
was significantly higher than the mean total protein value for the control horses.
Immediately following plasmapheresis, total protein of the treatment horses dropped to a
low of 6.20g/dl +/- 0.16g/dl, but remained within normal limits. Total protein
concentration of the eight treatment horses increased after the procedure in a linear
fashion, returning to baseline approximately 25 days after automated plasmapheresis.
Mean albumin concentration in the eight treatment horses was 3.11g/dl +/- 0.27g/dl prior
to automated plasmapheresis. Again, the baseline albumin concentration in the treatment
horses was significantly higher than the albumin concentration in the control horses.
Immediately following plasmapheresis, their albumin dropped to a low of 2.69g/dl +/-
0.20g/dl, but stayed within normal limits. Albumin concentrations increased after
automated plasmapheresis, but did not return to baseline within the 30 day post-
procedure study period. Mean IgG concentration in the treatment horses was 1,951mg/dl
+/- 220mg/dl before plasmapheresis. The baseline IgG concentration of the treatment
horses had a tendency to be greater than that of the control horses, but the difference was
not significant. Their IgG concentration decreased to 1,440mg/dl +/- 170mg/dl post-
plasmapheresis and increased in a linear fashion over the remaining study period. The
IgG concentration of the treatment horses returned to baseline approximately 29 days after automated plasmapheresis. Despite removing significant concentrations of IgG, the investigators did not observe an increase in frequency of disease or infection. The authors reported no significant changes in the percentage of albumin, α, β, or γ globulins as determined by electrophoresis over the course of the investigation. The authors did report significant increases in packed cell volume (PCV) post-plasmapheresis, which returned to baseline within 4 days after the procedure. In their discussion, the investigators attributed the increased baseline total protein, albumin and IgG concentrations of the treatment horses to increased synthesis and decreased catabolism in response to frequent bouts of hypoproteinemia. The authors compared the regularly plasmapheresed horses to regularly plasmapheresed human subjects, who have demonstrated maintenance of high serum protein concentrations. The investigators also hypothesized how donors were able to maintain normal protein concentrations during plasmapheresis and how they quickly replaced any deficits following the procedure. In addition to resynthesizing proteins, the authors suggested that the donors replaced the lost proteins from lymphatic and extravascular sources. The investigators attributed the transient increase in PCV following plasmapheresis to incomplete rehydration with lactated Ringer’s solution and stress induced splenic contractions. The authors clearly emphasized that the limiting factor of plasmapheresis is the rate of protein replacement following a procedure. They concluded that the protein recovery time corresponded well with the commercial program’s 30-day interval between plasmapheresis episodes.

In 2005, investigators from Switzerland designed a study to determine the effect of automated equine plasmapheresis on the behavior, general condition, hematological,
biochemical and coagulation variables in donor horses (Feige K, Ehrat F, Kastner S, et al., 2005). Swiss Warmblood horses, 3 geldings and 3 mares, aged 3-11 years and weighing 526kg +/- 43kg were used as donors. The six horses were clinically healthy with hematological and biochemical variables within normal ranges. None of the horses had experienced automated plasmapheresis prior to inclusion into the study. For the investigation, the horses were plasmapheresed with a Fresenius instrument (Blutzell-Separator AS 104, Fresenius, Stans, Switzerland) until 20ml of plasma/kg body weight were collected (approximately 10L of plasma per horse). This volume of plasma removal was selected because it is used frequently in commercial equine plasma production establishments and was used in previous studies with no observable adverse effects. During the procedure, plasma was replaced by an equal amount of lactated Ringer’s solution along with the donors’ blood cells. After automated plasmapheresis, the horses were evaluated at regular intervals for 31 days. The investigators determined that automated plasmapheresis was well tolerated by a majority of the donors. Only mild restlessness and headshaking was observed during the procedure. Results of physical examinations conducted immediately after plasmapheresis and over the subsequent 31 days were within normal limits. Immediately following automated plasmapheresis, the donors experienced a transient but significant increase in hematocrit, hemoglobin concentration, erythrocyte count and total leukocyte count. The investigators suggested that stress-related epinephrine release could have been responsible for the concomitant cellular increases. They also considered fluid shifting from the intravascular space to the extravascular space due to decreased oncotic pressure because of plasma protein loss as a cause for the increases. Total plasma protein concentrations decreased significantly from
6.35g/dl +/- 0.28g/dl before plasmapheresis to 5.22g/dl +/- 0.33g/dl after plasmapheresis. The total protein concentrations returned to the reference interval within three weeks of automated plasmapheresis, but did not return to baseline values over the length of the study. The investigators compared the behavior of total protein in this study to results of the study published in the American Journal of Veterinary Research, which saw a return to baseline in 25 days (Magdesian K, Brook D, Wickler S, 1992). The authors explained that the donors in the present study were naïve to the procedure, while the donors in the previous study were routinely plasmapheresed. The phenomenon of increased protein synthesis and decreased protein catabolism associated with repeated plasmapheresis had not occurred in these first-time donors. Therefore, they required a longer period of time to replace the lost plasma proteins. The authors also reported a significant drop in albumin post-plasmapheresis. While the decrease in albumin was significant, the concentrations remained within the reference interval. However, the investigators warned that even slightly decreased intravascular albumin levels indicate probably markedly emptied extravascular albumin stores, which may take 30-50 days to replenish. Biochemical variables affected by plasmapheresis returned to reference intervals quickly after the procedure. The authors did not express concern regarding any transient biochemical alterations. Clotting times and coagulation factors underwent only minor changes as a result of plasma collection. For 1-2 days after plasmapheresis there was a mild decrease in the activity of factor V, factor VIII and antithrombin, with a corresponding mild increase in the activated partial thromboplastin time (aPTT). The authors decided that these minimal changes were not clinically relevant and that harvesting 20ml plasma/kg body weight did not increase the donors to potential
coagulopathies. Overall, the investigators concluded that automated plasmapheresis was well tolerated by the donors. Changes in the hematological, biochemical and coagulation variables as a result of plasmapheresis were considered mild and had no clinical relevance for the donor horses.

**Human Plasmapheresis:**

As mentioned, there are few published reports that examine automated equine plasmapheresis. To this author’s knowledge, findings from the studies discussed in this publication represent all of the recent work that has been done in the investigation of equine plasmapheresis. While there is very little literature that focuses on automated equine plasmapheresis, a substantial number of investigations have evaluated the effects of automated human plasmapheresis (Bechtloff S, Tran-My B, Haubelt H, et al., 2005, Ciszewski T, Ralston S, Acteson D, et al., 1993, Hellstern P, Bach J, Haubelt H, et al., 2001, Tran-Mi B, Storch H, Seidel K, et al., 2004). Recently, a majority of human plasmapheresis research has focused on the impact that intensity of serial donor plasmapheresis has on source plasma quality and on donor health. Perhaps, findings from these investigations can shed some light on the unknowns of equine plasmapheresis.

To evaluate the impact that intensity of donation has on harvested plasma, a group of investigators from Germany examined plasma collected according to United States guidelines and plasma collected according to German guidelines (Hellstern P, Bach J, Haubelt H, et al., 2001). The investigators purchased 75 units of United States plasma harvested from regular human donors and 75 units of German plasma harvested from regular human donors. Plasma from both countries was collected with Haemonetics instruments (Haemonetics Corp, Braintree, MA) using 4% citrate anticoagulant at an
anticoagulant to blood ratio of 1:16. The median interval between plasma donations in the United States was 5 days, with a median collection volume of 875ml of plasma. The median interval between plasma donations in Germany was 14 days, with a median collection volume of 720ml of plasma. Significantly more plasma was harvested more frequently in the United States. Therefore, plasma from the United States represented product harvested from an intensive program, while plasma from Germany represented product harvested from a moderate program. When comparison was made of the source plasma, investigators determined that product harvested from a moderate program contained significantly higher concentrations of total protein, albumin and IgG. The most striking finding was the difference in IgG concentrations between the two groups. Plasma from the moderate plasmapheresis program contained 860mg/dl IgG, while plasma from the intensive plasmapheresis program contained 710mg/dl IgG. The concentration of IgG in plasma from the moderate program was 19.1% greater than the concentration in plasma from the intensive program. The investigators concluded that intensive automated plasmapheresis is safe because the total protein, albumin and IgG concentrations in the plasma from donors of the United States remained within normal limits. However, they questioned the effectiveness of the intensive program. Significantly more plasma was harvested in the United States, but that plasma contained markedly less total protein, albumin and IgG. With IgG as the driving force behind plasma collection, the investigators expressed concern over harvesting plasma containing lower concentrations of IgG. They suggested that further research was needed and should be aimed at optimization of the IgG yield.
The findings from the German study are in agreement with results from a small study evaluating Canadian human plasmapheresis (Ciszewski T, Ralston S, Acteson D, et al., 1993). Canadian programs harvest 500-600ml of plasma per session every 2-3 weeks. The study compared individuals who donated according to Canadian guidelines with a group who donated the same volume weekly. The investigators found lower total protein and IgG concentrations in the group that donated weekly. However, all of their values remained within normal intervals.

A study published in 2004 that evaluated the impact of different intensities of repeated plasmapheresis reported conflicting results (Tran-Mi B, Storch H, Seidel K, et al., 2004). Three different donor groups and a control group were used in the cross-sectional study. Human donors were plasmapheresed according to study on intensified plasmapheresis (SIPLA) guidelines, German national guidelines (GG) or council of European recommendations (CER) for a minimum of 12 months prior to the investigation. SIPLA donors were pheresed a median of 55 times and donated a median of 37L over the 12 months before they were evaluated. GG donors were pheresed a median of 25 times, donating a median of 16L. CER donors were pheresed a median of 16 times and donated a median of 10L in the 12 months leading up to the study. All three donor groups had significantly lower total protein, albumin and IgG concentrations in comparison to the non-donors. However, the intensity of donor plasmapheresis did not affect these variables, as there were no significant differences among the three donor groups. The investigators also measured specific IgG titers in donors and non-donors. Despite the lower IgG concentration in donors, there were no significant differences in IgG titers among the four study groups. The investigators reported no significant changes
in donor cellular immunity or iron stores. They also found no significant differences between donors and non-donors in regard to cardiovascular risk factors. The investigators concluded that intensive serial plasmapheresis programs appear as safe as moderate plasmapheresis programs, with respect to the variables they analyzed. Because the intensive programs seemed safe, the authors suggested conducting a prospective study to determine why donors withdraw from such plasmapheresis programs.

The authors took their own advice and published a prospective study on the safety of long-term intensive human plasmapheresis (Bechtloff S, Tran-My B, Haubelt H, et al., 2005). The investigators selected a group of serial plasmapheresis donors who participated in a moderate program and switched them to an intensive program. Before inclusion into the study, the donors gave between 35-38 units of plasma yearly with a minimum 48-hour interval between donations. After switching to the intensive program, the subjects were permitted to donate 60 units of plasma yearly with a minimum 72-hour interval between donations. Plasmapheresis was performed with a Baxter instrument (Autopheresis-C A 200, Baxter, Unterschleissheim, Germany) using 4% citrate at an anticoagulant to blood ratio of 1:16. Adverse events during donation and reasons for excluding subjects from donation were documented. The donors participated in the intensive plasmapheresis program over a three-year study period. A questionnaire was used to interview any dropouts during the investigation. At baseline, the donors had significantly lower total protein, albumin and IgG levels in comparison to non-donor controls. However, subsequent intensive plasmapheresis over three years did not cause any significant changes in these variables. Also, the investigators observed no marked or unexpected changes in red cell values, iron metabolism, cardiovascular risk factors or
coagulation variables over the study period. Information provided by the study’s questionnaire revealed that withdrawal from the intensive plasmapheresis program was predominantly related to socioeconomic issues or medical reasons not related to plasma donation. The investigators concluded that reasons for donor dropout from intensive plasmapheresis are not directly related to plasma donation itself.

A prospective multicenter study on the safety of long-term intensive human plasmapheresis was published in 2006 (Schulzki T, Seidel K, Storch H, et al., 2006). The investigators found that dropouts were predominantly due to socioeconomic or medical reasons unrelated to plasma donations. However, the occurrence of adverse events related to plasmapheresis was documented during the investigation. Severe adverse events, which required major medical intervention or hospitalization, were documented in 5 of 304,836 donations. They included four venipuncture adverse events and one metacarpal fracture after dizziness and fall. Adverse events, which required no or minimal medical intervention, were documented in 132 of 304,836 donations (0.04% of donations). These events included palpable hematomas and/or bleeding from venipuncture sites, citrate reactions, nausea and/or vomiting and dizziness. Despite the occurrence of adverse events related to the procedure, the investigators concluded that long-term intensive donor plasmapheresis is safe, provided that donors are carefully monitored.

The recently published reports on human plasmapheresis provide valuable information and insight into equine plasmapheresis. A review of the current literature indicates that human donors experience a sustained decrease in protein values in comparison to non-donor controls (Bechtloff S, Tran-My B, Haubelt H, et al., 2005, Tran-Mi B, Storch H, Seidel K, et al., 2004). These findings contradict earlier reports
that cited human and equine donors as having increased protein levels, which were thought to be the result of increased synthesis and decreased catabolism due to frequent bouts of hypoproteinemia (Branda R, 1978, Magdesian K, Brook D, Wickler S, 1992). Because of the conflicting results, it would be valuable to evaluate plasma protein concentrations in donor horses throughout their involvement in a plasmapheresis program. The current human literature also yielded interesting results in regard to the impact of plasmapheresis intensity. When considering donor health, investigators agreed that intensive serial plasmapheresis seemed safe (Bechtloff S, Tran-My B, Haubelt H, et al., 2005, Hellstern P, Bach J, Haubelt H, et al., 2001, Schulzki T, Seidel K, Storch H, et al., 2006, Tran-Mi B, Storch H, Seidel K, et al., 2004). However, some reports concluded that in comparison to moderate programs, intensive programs produced plasma with significantly lower protein concentrations (Ciszewski T, Ralston S, Acteson D, et al., 1993, Hellstern P, Bach J, Haubelt H, et al., 2001). In contrast, other studies reported no differences in protein concentrations in the comparison of plasma harvested from intensive plasmapheresis programs with plasma harvested from moderate plasmapheresis programs (Bechtloff S, Tran-My B, Haubelt H, et al., 2005, Tran-Mi B, Storch H, Seidel K, et al., 2004). The issue is quite controversial, with different countries adopting different guidelines that govern plasmapheresis schedules (Tran-Mi B, Storch H, Seidel K, et al., 2004). Currently, there is very little known about the impact of plasmapheresis intensity in equine donors. The literature has only reported investigations with repeated equine plasmapheresis at 30-day intervals (Magdesian K, Brook D, Wickler S, 1992). The effect of more intensive serial plasmapheresis programs with equine donors is unknown. From the results of human studies, one would hypothesize
that intensive equine plasmapheresis is safe. However, due to the conflicting data with human subjects, it is difficult to hypothesize the effect of intensive equine plasmapheresis on the harvested plasma. It would be beneficial to evaluate the effect of intensive equine plasmapheresis on donor health and on plasma quality.

Research Objectives:

Many questions remain unanswered regarding equine plasmapheresis. Results from recently published reports on human plasmapheresis provide insight, but not answers to the questions. Therefore, the goal of this research endeavor is to investigate some of the unknowns of intensive serial automated equine plasmapheresis. There are three main objectives of this research initiative. First, with Baxter instruments (Autopheresis-C A 200, Baxter-Fenwal, Lake Zurich, IL), design a continuous-flow, automated equine plasmapheresis procedure to harvest high-volume plasma according to cGMPs. After development of a repeatable and reliable procedure, create an operator’s manual to guide other investigators through each step of equine plasmapheresis. And third, perform relatively intensive serial plasmapheresis on equine donors and evaluate its effect on plasma quality and donor health.
CHAPTER II

Description of a high-speed, continuous-flow, automated equine plasmapheresis procedure

Abstract

Objective: The goal of this report was to develop and describe a high-speed, continuous-flow, automated plasmapheresis procedure for the collection of high-volume equine plasma in accordance with current good manufacturing practices (cGMPs).

Design: Donor horses were plasmapheresed at 14-day intervals with a maximum 22ml of plasma per kg donor body weight harvested during each plasmapheresis donation.

Animals: Horses included for the investigation were 3-10 years of age at the time of purchase. Draft breeds were preferred because of their docile temperament and large total blood volume.

Procedures: Adaptations were made to Baxter automated plasmapheresis instruments and Baxter sterile disposable collection sets, which allowed for dual-instrument, continuous-flow operation. Donor horses were connected to the apparatus via two jugular catheters. The instruments removed whole blood from the donors, fractionated the whole blood, diverted the plasma to collection bags and simultaneously returned the concentrated cells to the donors.

Results: Three thousand two hundred forty (3,240) plasmapheresis donations were performed, 143 different horses were plasmapheresed and more than 50,000L of sterile equine plasma were collected during the investigation. Donors remained calm during
plasmapheresis and tolerated the procedure. The high-volume and relatively frequent collections did not result in sustained hypoproteinemia in the donors. Adverse events were rarely observed as a result of the automated plasmapheresis technique. Each of the events occurred with extremely low frequency and the reoccurrence of events was minimized further by making minor adjustments to the procedure.

Conclusions and Clinical Relevance: The designed automated plasmapheresis procedure could be used to safely collect valuable equine plasma or to perform therapeutic equine plasmapheresis.

Abbreviations

- cGMPs: Current good manufacturing practices

Introduction

Equine plasma has been recognized as a valuable resource for well over one hundred years. In the early twentieth century, people with diphtheria infections were treated with plasma from horses immunized with diphtheria toxin, which provided passive immunization in the recipients (Abbas A, Lichtman A, 2005). Today, equine plasma is harvested and incorporated into culture media used by diagnostic laboratories. It serves as the matrix in pharmacokinetics studies that determine the half-lives of therapeutic compounds. Highly specific antibodies used in scientific assays are isolated from equine plasma. Plasma is harvested from equine donors for transfusion into equine patients for the management of protein-losing enteropathies, nephropathies, coagulopathies, failure of passive transfer and other medical conditions. Hyperimmune equine plasma is commercially manufactured and sold for prophylaxis and treatment of several clinically significant equine infectious diseases (Brook D, 1989, Magdesian K,
Brook D, Wickler S, 1992, Porter M, Green E, 2003). Equine plasma is the starting material in the production of hyperimmune equine Fab and F(ab)₂ products, which serve as vital pharmaceuticals in the human healthcare industry. These products neutralize viruses, toxins and venoms and are often the only treatment available in combating life-threatening medical conditions.

Different techniques have been employed over the last century to collect plasma from equine donors. Initially, investigators simply relied on the rapid erythrocyte sedimentation rate of equine whole blood to harvest the plasma (Feige K, Ehrat F, Kastner S, et al., 2003, Levine L, Broderick E, 1970, Morris D, 1999). They aseptically placed large bore stainless steel needles into jugular veins of equine donors. Tubing sets were attached to the needles and the donors’ whole blood was harvested into sterile glass collection jars containing anticoagulant (Porter M, Green E, 2003). Approximately 10-20% of the donors’ blood volume was removed, which resulted in an anticoagulant to whole blood ratio of 1:10 (Green E, Ward G, 1974, Porter M, Green E, 2003). Following whole blood collection, the glass jars were refrigerated for 12-24 hours to allow for gravity sedimentation. After the cellular mass separated to the bottom of the collection vessels, plasma was removed using a siphon or other technique. Once plasma was isolated, the remaining cellular elements were discarded. These early investigators performed the procedure without complication to donors approximately once every 30 days, yielding 2.5-5L of plasma per 500kg adult horse (Green E, Ward G, 1974, Morris D, 1999, Porter M, Green E, 2003).

In the 1970s, two separate teams of investigators described the technique of manual equine plasmapheresis for collection of horse plasma (Levine L, Broderick E,
1970, Green E, Ward G, 1974). The process involved the sequential steps of phlebotomy, fractionation, plasma extraction, resuspension of the cellular mass and return of the resuspended mass to the respective donors (Bartges J, 1997). The whole blood was harvested using techniques from the past. However, following 12-24 hours of gravity sedimentation and plasma collection, the concentrated cellular elements were resuspended in a volume of isotonic saline similar to the volume of plasma collected. The cells and saline were warmed in a water bath to 37°C and then transfused back into the donors. The process was quite lengthy with even more time required for cellular resuspension and transfusion. Overtime, investigators began to harvest the whole blood in bags, which were centrifuged to shorten significantly the time required for fractionation. Both teams of investigators performed manual plasmapheresis at weekly intervals without complication to donors, yielding 10-20L of plasma per 500kg horse each month (Levine L, Broderick E, 1970, Green E, Ward G, 1974).

The introduction of automated in-line blood cell separators in the early 1980s revolutionized the plasmapheresis procedure (Bartges J, 1997, Klages C, 2006). These instruments attached to the donors’ catheters via sterile disposable collection sets. Instrument pumps removed whole blood from donors and infused anticoagulant into the extracted blood at a controlled rate. The anticoagulated whole blood was then fractionated by either centrifugation or filtration techniques (Bartges J, 1997, Feige K, Ehrat F, Kastner S, et al., 2003, Morris D, 1999). Isolated plasma was diverted to attached collection bags or collection bottles. Simultaneously, the concentrated cells were returned to the donors. Some of the automated instruments provided fluid replacement during collection and others provided fluid replacement after plasma

Automated equine plasmapheresis offers advantages over previously used plasma collection methods. Automated plasmapheresis harvests plasma in a closed system, whereas the manual procedures harvest plasma in an open system. Open collection systems allow for possible bacterial contamination of plasma (Feige K, Ehrat F, Kastner S, et al., 2003, Morris D, 1999). Automated equine plasmapheresis is a more efficient collection method, harvesting a greater volume of plasma and returning cells in a shorter period of time (Feige K, Ehrat F, Kastner S, et al., 2003, Klages C, 2006). With automated plasmapheresis, blood is processed through sterile disposable tubing sets and separation devices. Because equipment is not used on multiple donors, the risk of cross-contamination is eliminated and there is no need for autoclaving or sterilizing materials prior to use (Klages C, 2006). The automated procedure also minimizes the extracorporeal deficit by simultaneously harvesting plasma and returning blood cells (Bartges J, 1997). Results from a study that evaluated equine plasma collection methods showed nearly complete removal of erythrocytes and leukocytes from plasma collected via automated centrifugation (Feige K, Ehrat F, Kastner S, et al., 2003). In comparison, a significant concentration of erythrocytes and leukocytes remained in product collected via both gravity sedimentation and blood bag centrifugation. Also, plasma collected by the automated technique demonstrated greater activity in factor VIII in comparison with plasma collected by gravity sedimentation. The few published reports on automated

While automated equine plasmapheresis represents a superior technique for the harvest of equine plasma, there is little information available with details of the collection procedure. Companies that manufacture plasmapheresis instruments market machines specifically for use with human donors, not equine donors. As a result, numerous logistical obstacles must be overcome before a significant volume of equine plasma can be collected by use of the instruments. Operating manuals that accompany the machines do not address the needs of automated equine plasmapheresis. Moreover, the sterile disposable collection sets and other supporting materials are designed for collection of 500-800ml of human plasma, not 20L of equine plasma. The three published reports from studies that investigated automated equine plasmapheresis briefly describe their collection techniques and indicate the instruments used for performance of the procedures (Feige K, Ehrat F, Kastner S, et al., 2003, Feige K, Ehrat F, Kastner S, et al., 2005, Klages C, 2006, Magdesian K, Brook D, Wickler S, 1992). The publications provide valuable information regarding automated equine plasmapheresis, but insufficient detail to replicate the procedures.

The goal of this research endeavor was to develop and describe a closed-loop, high-speed, continuous-flow, automated equine plasmapheresis procedure that collected high-volume source plasma according to cGMPs. Once a reliable and repeatable design

**Materials and Methods**

All research and development procedures were conducted with approval by the institutional animal care and use committee of Auburn University.

**Animals.** Horses included for the plasmapheresis investigation were 3-10 years of age at the time of purchase. Draft breeds were preferred over light breeds because of their docile temperament and large total blood volume. Horses arriving at the collection facility immediately entered pasture isolation for a minimum 30-day quarantine period. All horses were examined physically and implanted with a microchip for primary identification purposes. Blood samples were collected and submitted to establish baseline blood counts and serum biochemistry values. Separate blood samples were collected and submitted to test for equine infectious anemia via agar gel immunodiffusion and equine herpes virus-1 via polymerase chain reaction. While in quarantine, the horses were administered the anthelmintic moxidectin and vaccinated against Eastern equine encephalomyelitis, Western equine encephalomyelitis, West Nile virus, tetanus, rabies,
equine influenza, equine herpes virus-1, equine herpes virus-4e and Streptococcus equi. During the period of investigation, horses were maintained on mixed grass pastures with access to water and alfalfa hay ad libitum. In addition, approximately 2kg of pelleted grain were provided per horse daily.

**Autopheresis-C Instruments and Ohaus Scales.** Prior to performance of automated equine plasmapheresis, a number of modifications were made to the Autopheresis-C A-200 instruments. The attached weigh scale hanger associated with each Autopheresis-C instrument was permanently removed because the weight of plasma collected during equine plasmapheresis exceeded the capabilities of the instrument’s internal scale. Instead, an external Ohaus scale was used to weigh product during equine plasma collection. Switch #8 of the DIP switch S1 located on each instrument’s CPU board was placed in the “on” position. This prevented the “CK Blood Flow” message from being repeatedly displayed throughout the procedure. And, version APA 2.0 software for veterinary applications was installed on each Autopheresis-C instrument. Two modified Autopheresis-C instruments were used simultaneously per donor horse during equine plasmapheresis. The two instruments were positioned side-by-side at the head of each collection station. The pressure cuff of each instrument was not used on the donor during equine plasma collection, but instead was wrapped around a portion of the adjacent stocks. The Ohaus scale used to weigh product during equine plasmapheresis was positioned next to the Autopheresis-C instruments.

**Modified Collection Sets.** Two Baxter sterile disposable collection sets designed specifically for this investigation were adapted into one modified set for dual-instrument, continuous-flow operation. Adaptations were made using a medical tubing
sealer, a medical tubing welder, three pieces of custom-designed tubing and a custom-designed 20L collection bag. All adaptations were performed in a manner to maintain sterility of components. Initially, the two Baxter collection sets were removed from their packaging and oriented on a work top surface. By use of the medical tubing sealer, the cell line of each collection set was heat sealed where it entered the reservoir. The saline line of each set was sealed approximately 30-35cm from the spiked end. The spiked end of each sealed saline line was then separated from the set and discarded.

Next, the donor line of one set was welded to one 90cm long line of EPS3 custom tubing. The procedure was repeated with the remaining donor line and the remaining 90cm long line of EPS3 tubing. Next, each sealed cell line was welded to each 90cm long line of EPS1 custom tubing. Then, the P2 line of both collection sets was lengthened by welding approximately 45-50cm of sterile tubing into the line. The modified set was installed onto the side-by-side Autopheresis-C instruments. Once the modified set was installed onto the instruments, each 70cm long line of EPS2 tubing was welded to each plasma line. The remaining 90cm long piece of EPS2 tubing was welded to the centrally located port of the EPS-20L collection bag. The 20L bag was slipped into a protective liner and placed on top of a cool pack resting at the bottom of a Nalgene tub. The tub and its contents were set on the Ohaus scale in order to weigh plasma collected in the 20L bag.

**Donors.** Donor horses consisted of 88 mares and 55 geldings (98 Belgians, 30 Percherons, 4 Standardbreds, 3 Warmbloods, 2 Percheron crosses, 2 Belgian crosses, 2 Standardbred crosses, 1 Paint cross and 1 Shire cross) aged 4 to 14 years (8 +/- 2 years) and weighing 804 +/- 97kg. Horses entered the plasmapheresis schedule based on achieving benchmark antibody titers. Potential donors were identified from the schedule.
and a record review was conducted for each horse. Any horses placed on an established medical deferral were determined unsuitable for collection. All suitable donor horses were brought to the treatment barn for examination and approval. Each donor was verified by primary identification, weighed and physically examined. A rectal temperature was obtained using a National Institute of Standards and Technology traceable certified thermometer. A large rectangle of hair was clipped from over each jugular vein. One vacuum blood tube containing ethylene diamine tetraacetic acid (EDTA) and one vacuum blood tube containing no anticoagulant were collected from each suitable donor. Plasma from EDTA anticoagulated blood was used to determine total plasma protein concentration for each horse via refractometry. Healthy horses with rectal temperatures less than 39.5°C and total plasma protein concentrations greater than 5.5g/dl were accepted for plasmapheresis. Accepted donor horses were mildly sedated with intravenous 2.5-7.5mg detomidine\(^9\) and led into the collection building. Each donor was guided into a set of stocks and secured with butt and back ropes. The horses’ heads were situated in slings made from padded saddle girths, while their chests rested against the doors of the stocks. Previously positioned Autopheresis-C instruments and Ohaus scales were located near the right shoulder of each donor. The clipped area over each jugular vein was prepared for catheterization with 2% chlorhexidine acetate\(^9\) and a one-step antiseptic sponge\(^7\). Approximately 1.5ml of 2% lidocaine HCl\(^8\) was injected subcutaneously over each catheterization site and a small skin incision was produced in the anesthetized areas with a #15 blade. Ten-gauge, 76mm catheters\(^4\) were inserted through each skin incision and directed ventrally into each jugular vein of donor horses. The catheters were capped and secured into place with 2-0 sutures\(^u\).
Equine Plasmapheresis. The luer end of the EPS3 tubing line was connected to each donor’s right jugular catheter, while the luer end of the EPS1 tubing line was connected to each donor’s left jugular catheter. Both tubing lines were secured to the donors’ necks with sutures. A tourniquet was then wrapped around the neck distal to the catheterization sites of each donor to prevent jugular vein collapse during plasmapheresis. Following tourniquet placement, both Autopheresis-C instruments were advanced simultaneously to initiate the priming sequence, which occurred at the start of every procedure. Once priming was complete, both instruments performed collection and reinfusion cycles concurrently. Whole blood was extracted from each donor’s right catheter and entered the EPS3 tubing line. The line divided and delivered blood to both donor lines of the modified Plasmacell-C set. Four percent anticoagulant sodium citrate solution was infused into the whole blood, which resulted in an anticoagulant to whole blood ratio of 1:16. This ratio was achieved by programming the instrument to operate at an anticoagulant setting of 6%. Next, the anticoagulated whole blood traveled toward each Autopheresis-C instrument via the blood lines. The blood pump sent anticoagulated blood to the separation device, which acted as a rotating membrane filter to separate plasma from cellular components of blood. Plasma exited the bottom port of the separation device, passed through the refractometer and entered the 20L collection bag. Simultaneously, concentrated blood cells exited the side port of the separation device, traveled through the cell pump and immediately back to the donor via the left jugular catheter. The reservoir was completely bypassed, which eliminated the need for intermittent collection and reinfusion cycles. The plasmapheresis procedure was terminated based upon the following conditions: reaching a 22ml of plasma per kg donor
body weight target, reaching the 20L maximum capacity of the bag, if the donor’s health was in jeopardy, if the instruments were unable to maintain plasma production, in the event of facility power loss or at the project director’s request. The procedure was ended by pressing the stop button on both instruments. The modified collection set was sealed along both plasma lines, both anticoagulant lines, the EPS1 line and the EPS3 line. This freed the donor and plasma collection bag from the disposable collection set, which was discarded as medical waste. The plasma collection bag was transported to a separate room for further processing. There, the plasma was homogenized and steriley aliquoted into 1L high density polyethylene bottles. Appropriate labels were applied to the bottles, which were stored at a set temperature of -35°C in a continuously monitored freezer.

The donor horses were closely observed throughout plasmapheresis. Pulse rate, respiratory rate, mucous membrane evaluation and electrocardiogram were recorded approximately mid-procedure and at the end of plasmapheresis. Donors were administered 2.5-7.5mg detomidine or 2.5-7.5mg detomidine and 2.5-7.5mg butrophanol as needed to prevent restlessness and excessive movement during plasmapheresis. Sedatives were administered intravenously through an infusion port located in the EPS1 tubing line. Once the plasmapheresis procedure ended and the tourniquet was removed, each donor received 15L of intravenous fluids via gravity flow. Jugular catheters were removed after fluid administration, with hemostasis achieved using 4 x 4 gauze sponges and slight pressure. The donors were washed down, visually inspected and returned to their designated pastures.
**Statistical Methods.** Reported in this publication are a case incident and the frequency of adverse events, which were determined without a statistical software package. Using SAS software, Fisher’s Exact One Tail test was performed to determine if there was a significant difference (p < 0.05) between two of the reported frequencies.

**Results**

The equine plasmapheresis technique described in Materials and Methods has been used since 2005 to perform more than 3,200 plasmapheresis episodes. Performed at 14-day intervals, a maximum of 22ml of plasma was harvested per kg donor body weight. Horses weighing 910kg and greater donated a maximum 20L of plasma, which was the collection bag capacity. Donation sessions typically lasted between 3-6 hours. Over the past five years, 143 different donors were plasmapheresed, yielding more than 50,000L of sterile equine plasma collected according to cGMPs.

Serial automated equine plasmapheresis according to the described technique and schedule was well tolerated by a majority of the donor horses. Not a single donor was determined unacceptable for plasmapheresis due to a total plasma protein concentration that dropped below 5.6g/dl. Most donors remained relatively calm during the procedure, with only mild excitement or head shaking. While a majority of the donors tolerated plasmapheresis and became more comfortable overtime, a few donors repeatedly resented the procedure. Conditioning and multiple plasmapheresis sessions did not improve the tolerance of these few horses. These donors exhibited moderate to severe head shaking, stomping, striking, biting and stock jumping.

Several adverse events related to equine plasmapheresis also were observed. The inadvertent subcutaneous return of concentrated blood cells was recognized as a firm
perijugular swelling greater than 5cm x 5cm, which occurred in association with the jugular vein that received concentrated blood cells. Subcutaneous return of concentrated cells occurred during twenty-two plasmapheresis procedures, corresponding to a frequency of 0.7% (22/3240). Development of excessive scar tissue, defined as the formation of scar tissue greater than 4cm in diameter at the site(s) of previous catheter insertion, was documented as an adverse event. One of the 143 donors developed marked scar tissue formation, reflecting an incidence proportion of 0.6%. Increased respiratory rates were recognized as an adverse event when rates reached \(\geq 30\) breaths per minute and continued to increase for sustained periods, which resulted in premature stoppage of the plasmapheresis procedure. Twenty-two of the 3,240 plasmapheresis donations were prematurely terminated because of increased respiratory rates, which corresponds to a frequency of 0.7% (22/3240). Immediately following plasmapheresis and tourniquet removal, eleven different donors demonstrated one or multiple neurological abnormalities on a single occasion (11/2850, 0.4%). These abnormalities included ataxia, inability to stand and unilateral or bilateral blindness. After making minor adjustments to the plasmapheresis and post-plasmapheresis procedures, the occurrence of these neurological abnormalities was eliminated (0/390, 0.0%). No significant difference was found when comparing the frequency of the events before and after changes were implemented (\(p = 0.24\)). Death after plasmapheresis donation occurred three times during the investigation (3/3240, 0.09%). Donors demonstrated weakness with resultant collapse within 20 minutes post-plasmapheresis and died within 12 hours of the plasmapheresis procedure. No deaths occurred during plasmapheresis throughout the investigation.
A high-speed, continuous-flow, automated plasmapheresis procedure was developed to collect high-volume equine plasma in a closed and sterile system. Performance of plasmapheresis on equine donors required modification of instruments and sterile collection sets that were designed for use on human donors. High-speed and high-volume equine plasmapheresis was accomplished by simultaneous operation of two modified Autopheresis-C instruments per one donor horse. Both instruments were programmed to process 135-150ml of whole blood each minute, with both instruments producing approximately 30-50ml of plasma each minute. A closed system was maintained through use of tubing sealers and welders to perform modifications made to the collection sets. Continuous-flow operation was achieved by bypassing the collection set reservoir and immediately returning concentrated blood cells to the donor. This modification allowed the instruments to simultaneously perform both collection and reinfusion cycles. Prior to introducing this set modification, the reservoir received concentrated cells, with the Autopheresis-C instrument alternating between collection and reinfusion cycles. A published report on automated caprine plasmapheresis described use of the Autopheresis-C instrument and alternating cycles to collect goat plasma (Klages C, 2006). Because the reservoir was utilized and cycles were alternated, they described an intermittent-flow plasmapheresis procedure.

To the author’s knowledge, this is the first report that describes automated equine plasmapheresis repeated at 14-day intervals. Prior to this report, a similar volume of plasma had been harvested from equine donors via automated plasmapheresis, but only at 30-day intervals (Feige K, Ehrat F, Kastner S, et al., 2003, Feige K, Ehrat F, Kastner S, et
The previous investigators recommended the 30-day interval between plasmapheresis donations to allow donor horses time to replenish depleted total protein, albumin and IgG concentrations. Despite performance of automated plasmapheresis with approximately twice the previously reported frequency, not a single equine donor was determined unacceptable for plasmapheresis due to total plasma protein concentration below 5.6g/dl. In fact, a total plasma protein concentration below 6.0g/dl was never recorded in over 3,200 donor approval examinations. It is reasonable to conclude that collecting up to 22ml of plasma per kg donor body weight every 14 days does not result in sustained hypoproteinemia.

In general, the automated plasmapheresis procedure was well tolerated by the equine donors. A majority of the horses stood relatively still during the 3-6 hours of plasmapheresis and required infrequent administration of sedation. In contrast, there were a few donors that repeatedly behaved poorly during plasmapheresis. These horses bit, struck, stomped or completely jumped out of the stocks, which occasionally resulted in premature stoppage of the plasmapheresis session. Attempts were made to condition these horses to the procedure. Hobbles were securely fastened around the forelimbs of horses known to jump or stomp during plasmapheresis. Horses that demonstrated dangerous behavior and posed a threat to employees were re-sedated more frequently. It was determined that these horses not only displayed this behavior during plasmapheresis, but also when they were administered anthelmintics, vaccinated, placed in stocks or handled for other reasons. Overall, these horses possessed poor temperaments and the recommendation was made to cull them from the program.
The three previously published reports on automated equine plasmapheresis did not describe any adverse events occurring as a result of the procedure (Feige K, Ehrat F, Kastner S, et al., 2003, Feige K, Ehrat F, Kastner S, et al., 2005, Klages C, 2006, Magdesian K, Brook D, Wickler S, 1992). In contrast, infrequent, but clinically important adverse events related to the automated plasmapheresis procedure were documented during this investigation. The adverse reactions ranged from minor issues, to grave events. Regardless of the severity of the reaction, each incident was documented, investigated and attempts were made to prevent incident recurrence.

Rarely, concentrated blood cells returned to donors were reinfused subcutaneously rather than intravenously. It was determined that this adverse event was related to inaccurate catheter placement or to catheter dislodgement as a result of donor head shaking. The plasmapheresis procedure was temporarily stopped and the donor was evaluated upon recognition of swelling over the jugular vein, which indicated the subcutaneous return of concentrated cells. If identified quickly, the donor was re-prepared and a new catheter placed in the original site. If swelling prevented use of the original site, a new catheterization site was selected above or below the previous site. Occasionally, the new catheter was placed in the opposite jugular vein, below the catheter removing whole blood for plasmapheresis. If the horse’s health appeared in jeopardy or if the catheter could not be replaced, the procedure was prematurely terminated. To prevent the incident from reoccurring, jugular catheters were always sutured into place at the insertion site. Following any problematic catheter placement, proper positioning of the catheter was confirmed by the ability to remove whole blood from the catheter with a syringe. The horses’ heads were situated in padded slings and cross ties were applied to
reduce head shaking during plasmapheresis. Catheter sites were also closely observed throughout the procedure for any unexpected swelling. While subcutaneous return of concentrated cells was recognized as an adverse event, it occurred with an extremely low frequency (22/3240, 0.7%) and did not cause further complications in the donor.

The appearance of scar formation was expected with jugular catheterizations occurring every 14 days, however, most sites healed quickly and obvious evidence of frequent catheterization was not readily apparent. The rapid healing and lack of gross scar formation was attributed to aseptic technique, skilled catheter placement and gentle catheter removal. Despite the success observed in most donors, one donor horse developed diffuse and mildly painful scars along both jugular furrows after repeated plasmapheresis. Subsequently, catheters were placed above or below the scar formation that developed in this donor horse. However, with serial plasmapheresis, the space to place catheters became limited and overtime it became necessary to insert catheters through the scarred tissue. This horse was observed with particular closeness during plasmapheresis because it became relatively difficult to accurately catheterize the veins. While the scar tissue that developed in the horse was problematic, its formation never resulted in the inability to perform plasmapheresis.

Increased respiratory rates were observed during equine plasmapheresis. The definitive cause of the increased rates was never determined, but several possibilities were considered, including: donor excitement, red blood cell lysis, temporary hypovolemia and undetected cardiac disease. The plasmapheresis session was allowed to proceed despite the increased rates. However, the procedure was halted and the donor evaluated if the rates continued to increase. If the rates remained increased and the
donor’s health was of concern, plasmapheresis was terminated. If the rates eventually decreased back within normal limits and no other clinical abnormalities were detected, plasmapheresis resumed without complication. To reduce donor excitement, visual and auditory stimuli were kept at a minimum in the collection room. To prevent red blood cell damage during plasmapheresis, care was taken to properly install tubing onto pump rollers and to protect tubing lines from crushing forces. To reduce occurrence of temporary hypovolemia, the speed of plasmapheresis was decreased in donors that demonstrated increased respiratory rates. Cardiac auscultation and electrocardiograms were performed on donors prior to and during plasmapheresis to identify cardiac abnormalities, but evidence of cardiac disease was not detected in any of these horses.

Following plasmapheresis and tourniquet removal, one or a combination of neurological abnormalities were observed in eleven separate donors on single occasions (11/2850, 0.4%). These donors demonstrated ataxia, inability to stand and unilateral or bilateral blindness. While these adverse events did not occur with significant frequency ($p = 0.24$), the gravity of the events was significant. A diagnosis of cerebral and cerebellar reperfusion injury was made based on clinical signs and after ruling out other potential causes. The event was attributed to inadvertent carotid artery occlusion, abrupt tourniquet removal and prompt administration of intravenous fluids post-plasmapheresis. Numerous adjustments were implemented to prevent reoccurrence of post-procedure reperfusion injury. The tourniquet used during plasmapheresis was redesigned to prevent carotid artery occlusion and employees were trained on proper tourniquet application. Tourniquet removal was adapted into a gradual process and no longer an abrupt event. Instead of administering intravenous replacement fluids immediately following
plasmapheresis, administration of fluids was delayed a minimum of ten minutes. After implementation of all changes, neurological abnormalities were no longer observed following plasmapheresis and tourniquet removal (0/390, 0.0%). The other published reports on equine plasmapheresis did not describe use of a tourniquet on their donors, which may explain why they did not observe signs associated with reperfusion injury. A published report on manual bovine plasmapheresis described use of a neck tourniquet to provide adequate blood flow during plasma collection (McVey D, Loan R, 1989). No adverse events associated with plasmapheresis were documented in these cattle, but only three subjects were used in the short-term study.

Three donors died within 12 hours of completing plasmapheresis donation. The donors demonstrated weakness with resultant collapse within 20 minutes post-plasmapheresis. After collapse, two donors had increased pulse rates with sustained ventricular tachycardia as determined by ECG. Necropsy results from one donor that experienced cardiac abnormalities revealed acute, multifocal, moderate myocardial necrosis. Other findings at necropsy from the three donors were unremarkable. While gross and histological findings at necropsy did not demonstrate evidence of cerebral or cerebellar reperfusion injury, no deaths related to plasmapheresis occurred after implementation of the changes to prevent reperfusion injury.

This report describes a safe and reliable method for repeatedly harvesting 22ml of plasma per kg donor body weight from accepted equine donors as frequently as every 14 days. Any adverse events related to the plasmapheresis procedure can be kept to a minimum by selection of donors with acceptable temperaments, careful catheterizations, close monitoring of donors during collection and prevention of the occurrence of
reperfusion injury. This method of automated plasmapheresis can be used to collect high-volume equine plasma according to cGMPs. Collected equine plasma has numerous uses in research and healthcare settings. This method could also be used to perform therapeutic plasmapheresis on equine patients, a form of treatment never reported in equine medicine. The theoretical basis behind therapeutic plasmapheresis is the removal of pathogenic materials from circulation (Bartges J, 1997). Presumably, these materials are proteins, protein-bound or high molecular-weight solutes and include: autoantibodies, circulating immune complexes, excess lipids or hormones, exogenous toxins and other abnormally occurring molecules. Therapeutic plasmapheresis has been used in human and canine medicine to manage autoimmune diseases, neoplasia, hyperviscosity, sepsis, intoxication and other conditions.

Footnotes

a. Autopheresis-C A-200, Baxter-Fenwal, Lake Zurich, IL
b. Quest Gel, Fort Dodge Animal Health, Fort Dodge, IA
c. West Nile Innovator + EWT, Fort Dodge Animal Health, Fort Dodge, IA
d. RabVac 3, Fort Dodge Animal Health, Fort Dodge, IA
e. Fluvac Innovator EHV-4/1, Fort Dodge Animal Health, Fort Dodge, IA
f. Pinnacle IN, Fort Dodge Animal Health, Fort Dodge, IA
g. Life Design Prime 14, Nutrena, Minneapolis, MN
h. Ohaus Champ Square CQ50L scale with CD33 indicator, Pine Brook, NJ
i. 4R-2252 Plasmacel set, Baxter-Fenwal, Lake Zurich, IL
j. Baxter AutoSeal Tubing Sealer, Baxter-Fenwal, Lake Zurich, IL
k. TSCD Sterile Tubing Welder, Terumo Medical Corporation, Somerset, NJ
l. 03-220-EPS1 tubing set, Charter Medical, Winston-Salem, NC
m. 03-220-EPS2 tubing set, Charter Medical, Winston-Salem, NC
n. 03-220-EPS3 tubing set, Charter Medical, Winston-Salem, NC
o. EPS-20L Collection Bag, Charter Medical, Winston-Salem, NC
p. Dormosedan, Pfizer Animal Health, Exton, PA
q. Nolvasan Surgical Scrub, Fort Dodge Animal Health, Fort Dodge, IA
r. ChloraPrep One-Step Antiseptic Sponge, Medi-Flex Hospital Products, Overland Park, KS
s. Hospira, Inc., Lake Forest, IL
t. BD, Franklin Lakes, NJ
u. Supramid, S. Jackson, Inc., Alexandria, VA
v. Baxter-Fenwal, Lake Zurich, IL
w. Plasmalink Pooling Bottle, Baxter-Fenwal, Lake Zurich, IL
x. Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA
y. Plasma-Lyte A, Baxter-Fenwal, Lake Zurich, IL
CHAPTER III

The effects of serial plasmapheresis on total plasma protein and total immunoglobulin G concentrations in donors throughout their involvement in an equine plasmapheresis program

Abstract

Objective: The goal of this study was to determine the effects of intensive serial plasmapheresis on total plasma protein and total IgG concentrations in donors throughout their involvement in an equine plasmapheresis program.

Design: Retrospective Study. Evaluate horses that donated 22ml of plasma per kg donor body weight at 14-day intervals a minimum of eight consecutive plasmapheresis donations.

Animals: Thirteen mares and five geldings (13 Belgians, 3 Percherons, 1 Standardbred, 1 Warmblood) aged between 7 to 14 years (10 +/- 3) and weighing 822 +/- 128kg satisfied study criteria.

Procedures: Automated equine plasmapheresis was performed using two modified Baxter Autopheresis-C instruments per one donor horse. Plasma samples were collected at each donation for determination of total protein concentration and total IgG concentration. Total plasma protein concentrations were determined by refractometry. A commercially available ELISA (enzyme-linked immunosorbent assay) was used to determine total equine IgG concentrations.
Results: The eighteen different donor horses participated in 8-19 (13 +/- 3) serial donations during the investigation. The donor horses experienced statistically significant ($P < 0.05$) decreases in both plasma protein and IgG concentrations over the investigation period.

Conclusions and Clinical Relevance: The evaluated plasmapheresis protocol did cause statistically significant decreases in both plasma protein and IgG concentrations, however, decreases were not physiologically significant. In conclusion, performing automated equine plasmapheresis according to the described protocol did not result in sustained depletion of total plasma protein or total IgG concentrations.

Abbreviations

IgG Immunoglobulin G
ELISA Enzyme-linked immunosorbent assay
RID Radial immunodiffusion

Introduction

Equine plasma and its constituents are valuable and potentially life-saving resources used by diagnostic laboratories, researchers, veterinarians and physicians. Equine plasma may be incorporated into culture media or used as the matrix in pharmacokinetic studies. Highly specific antibodies may be isolated from equine plasma and used in production of scientific assays. Plasma is harvested from donors and infused into equine patients to manage protein-losing enteropathies, nephropathies, coagulopathies, failure of passive transfer and other medical conditions. Hyperimmune equine plasma is commercially manufactured and sold the prophylaxis and treatment of several clinically significant equine infectious diseases (Brook D, 1989, Magdesian K,
Equine plasma is the starting material used in production of hyperimmune equine Fab or F(ab)₂ products, which are commonly referred to as antivenoms and antitoxins in the human healthcare industry. These pharmacological agents are frequently the only treatment available in management of envenomations and bacterial intoxications.

Plasmapheresis is the technique currently used to harvest large volumes of equine plasma. Plasmapheresis involves removal of whole blood, addition of an anticoagulant, fractionation of anticoagulated whole blood, collection of plasma and return of cellular components back to the donor (Bartges J, 1997, Magdesian K, Brook D, Wickler S, 1992). Equine plasmapheresis was first performed by use of a manual technique. Two separate reports in the 1970s described similar methods for manual plasmapheresis of equine donors (Levine L, Broderick E, 1970, Green E, Ward G, 1974). Investigators aseptically placed large bore stainless steel needles into jugular veins of donors. Tubing sets were attached to the needles and the donors’ whole blood was harvested into sterile glass collection jars containing anticoagulant. Following 12-24 hours of refrigerated gravity sedimentation, the plasma fraction was siphoned into sterile collection jars. The remaining cellular mass was resuspended in a volume of isotonic saline similar to the volume of plasma collected. The cells and saline were warmed in a water bath to 37°C and then transfused back into the donors. Today, instruments are used by researchers and commercial operations to perform automated equine plasmapheresis. These instruments connect to the donors via sterile tubing sets, which forms a closed-loop collection system (Feige K, Ehrat F, Kastner S, et al., 2003). Automated instruments withdraw whole blood and infuse it with anticoagulant at a controlled rate. Anticoagulated whole blood is
fractionated by either centrifugation or filtration techniques (Bartges J, 1997, Feige K, Ehrat F, Kastner S, et al., 2003, Morris D, 1999). Isolated plasma is diverted to an attached collection bag or bottle. Simultaneously, the concentrated cells are returned to the equine donors.

Research facilities and commercial operations repeatedly plasmaphereze donor horses at 14-30 day intervals, and harvest a maximum of approximately 20ml of plasma per kg donor body weight during each collection (Feige K, Ehrat F, Kastner S, et al., 2003, Feige K, Ehrat F, Kastner S, et al., 2005, Klages C, 2006, Magdesian K, Brook D, Wickler S, 1992). While a number of facilities take advantage of automated equine plasmapheresis to harvest a large volume of sterile plasma, only a few studies have examined the procedure and its effects over time. One study evaluated the effects of automated equine plasmapheresis on clinical, hematological, biochemical and coagulation variables, but only after a single plasmapheresis donation (Feige K, Ehrat F, Kastner S, et al., 2005). Another study evaluated the replacement rate of serum proteins after plasmapheresis in eight donor horses (Magdesian K, Brook D, Wickler S, 1992). The horses used in the investigation belonged to a commercial plasmapheresis program and had previously undergone 6-18 plasmapheresis donations at a mean of once every 30 days prior to the investigation. The rate of protein replacement was only evaluated once and not throughout the donors’ involvement in the plasmapheresis program.

The purpose of collecting equine plasma is to harvest valuable proteins, including IgG. Equine plasma collection facilities aim to harvest high-volume, high-quality plasma as frequently as possible without causing sustained protein depletion in donors. The goal of this study was to determine the effects of intensive serial plasmapheresis on total
plasma protein and total IgG concentrations in donors throughout their involvement in an equine plasmapheresis program.

**Materials and Methods**

All research procedures were conducted with approval by the institutional animal care and use committee of Auburn University.

**Treatment Group.** Thirteen mares and five geldings (13 Belgians, 3 Percherons, 1 Standardbred and 1 Warmblood) aged 7 to 14 years (10 +/- 3 years) with a mean body weight of 822 +/- 128kg were used as donors. The horses were clinically healthy based on results of physical examination, complete blood counts and serum biochemistry values. The horses were negative for equine infectious anemia by agar gel immunodiffusion and for equine herpes virus-1 assayed by polymerase chain reaction. Horses were either naïve to the plasmapheresis procedure or had not undergone plasmapheresis for a mean of 132 +/- 32 days before participation in the study. During the period of investigation, horses were maintained on mixed grass pastures with access to water and alfalfa hay ad libitum. In addition, approximately 2kg of pelleted grain were provided per horse daily. While included in the investigation, horses were administered the anthelmintic moxidectin and vaccinated annually against tetanus, rabies, equine influenza, equine herpes virus-1, equine herpes virus-4 and Streptococcus equi. Horses were vaccinated twice annually against Eastern equine encephalomyelitis, Western equine encephalomyelitis and West Nile virus. Donor horses were also regularly immunized against a project-specific immunogen during the investigation period.
**Plasmapheresis.** Treatment horses were approved for automated plasmapheresis once every 14 days. Prior to the collection procedure, potential donors were weighed and physically examined. Horses accepted for plasmapheresis were physically healthy with rectal temperatures less than 39.5°C and total plasma protein concentrations greater than 5.5g/dl, as determined by refractometry. A large rectangle of hair was clipped from over each jugular vein of approved donors. The areas were prepared for catheterization using 2% chlorhexidine acetate and a one-step antiseptic sponge. Approximately 1.5ml of 2% lidocaine HCl was injected subcutaneously over each catheterization site and a small skin incision was produced in the anesthetized areas with a #15 blade. Ten-gauge, 76mm catheters were inserted through each skin incision and directed ventrally into both jugular veins of donor horses. The catheters were capped and secured into place with 2-0 sutures.

Plasmapheresis was performed with horses standing in stocks and their heads situated in padded saddle girths. Each donor’s catheters were connected to a modified Plasmacell-C set, which was installed on two simultaneously operating Baxter Autopheresis-C instruments. Tourniquets were wrapped around the donors’ necks to provide adequate blood flow during the procedure. Whole blood was continuously extracted from the outflow catheter and infused with sodium citrate solution at 6%, resulting in an anticoagulant to whole blood ratio of 1:16. The anticoagulated whole blood traveled through the modified Plasmacell-C set toward the separation device associated with each operating instrument. The separation device acted as a rotating membrane filter to separate plasma from cellular components of blood. Plasma exited the bottom port of each separation device, passed through the instrument’s refractometer and
entered the 20L-capacity collection bag\textsuperscript{9}. Simultaneously, concentrated blood cells exited the side port of each separation device, traveled through the cell pump and immediately back into the donor via the inflow catheter. The two instruments each processed approximately 135ml of whole blood every minute.

Automated equine plasmapheresis was terminated when any one of the following conditions was met: the target of 22ml of plasma per kg donor body weight was reached, the 20L maximum capacity of the plasma collection bag was reached, the donor’s health was in jeopardy, the instruments were unable to maintain plasma production, the facility’s power was lost or at the request of the project director. The procedure was ended by pressing the stop button on both instruments. Tubing of the modified Plasmacell-C set was heat sealed to maintain the closed collection system. This freed the donor and plasma collection bag from the disposable set, which was discarded as medical waste. The plasma collection bag was transported to a separate room for further processing. There, the plasma was homogenized, steriley aliquoted into 1L high density polyethylene bottles\textsuperscript{9}, labeled appropriately and stored at a set temperature of -35°C in a continuously monitored freezer.

Donor horses were closely observed throughout plasmapheresis. Pulse rate, respiratory rate, mucous membrane evaluation and electrocardiogram were recorded approximately mid-procedure and at the end of plasmapheresis. Donors were administered 2.5-7.5mg detomidine\textsuperscript{7} or 2.5-7.5mg detomidine and 2.5-7.5mg butrophanol\textsuperscript{8} as needed through an infusion port located in the modified Plasmacell-C set. Once the plasmapheresis procedure ended and the tourniquet was removed, each donor received 15L of intravenous fluids\textsuperscript{1} via gravity flow. Jugular catheters were removed
after fluid administration, with hemostasis achieved using 4 x 4 gauze sponges and slight pressure. The donors were washed down, visually inspected and returned to their designated pastures.

**Total Protein Determination.** The total plasma protein concentration was determined for each donor within 24 hours of the impending automated plasmapheresis procedure. A 7ml whole blood sample was collected from the jugular vein of each donor into a vacuum blood tube containing 12mg ethylene diamine tetraacetic acid (EDTA). Plasma samples from these tubes were used to determine the total plasma protein concentration via refractometry. One refractometer was used to measure all samples throughout the investigation. The refractometer was calibrated and compared against a known concentration each day before use.

**Total IgG Determination.** Plasma samples were obtained from each donor’s collection bag the day of plasmapheresis using a 3ml-capacity vacuum blood tube containing no additive. The samples were stored frozen at or below -20°C until IgG analysis, when the samples were thawed in a water bath at 37°C. Total equine IgG was determined using a commercially available ELISA kit, which was validated for repeatability and reproducibility. The horse IgG standard, derived from purified horse IgG, was validated for accuracy against a horse IgG reference serum. All samples and standards were analyzed in duplicate on an Ultra Microplate Reader with KC Junior software.

**Statistical Methods.** Statistical methods used include a mixed model for repeated measures analysis of variance and Dunnett’s test to compare means. Mean total protein and mean IgG concentrations determined for subsequent plasmapheresis
donations were compared to the mean concentrations determined at donation number one (baseline). A \( P \) value of <0.05 was used as the level of significance for hypothesis testing. Analysis was conducted using SAS software.

**Results**

The eighteen treatment horses were plasmapheresed a mean of once every 15 +/- 3 days and donated a mean of 21 +/- 1ml of plasma per kg body weight. The automated plasmapheresis donations lasted between 2-6 hours with most donation sessions 4-5 hours long. Donor horses participated in 8-19 (13 +/- 3) serial donations over the investigation period. None of the treatment horses were deferred from participation in plasmapheresis due to rectal temperatures \( \geq 39.5^\circ\text{C} \) or total protein concentrations \( \leq 5.5\text{g/dl} \). With the exception of one very restless and anxious horse, the plasmapheresis procedure was well tolerated by the donor horses. They remained calm and infrequently required administration of sedation during the collection procedure. Donors remained in good condition immediately following automated plasmapheresis and during intervals between plasmapheresis donations.

Mean total plasma protein concentration for treatment horses was 7.49 +/-0.59g/dl at the time of initial plasmapheresis (Table 3.1 and Figure 3.1). Mean total plasma protein concentration demonstrated an initial statistically significant decrease at the third plasmapheresis donation, at which time the concentration dropped to 7.12 +/- 0.34g/dl (\( P = 0.01 \)). Mean total plasma protein concentration remained significantly decreased through the fourteenth donation. The largest decrease in mean total plasma protein concentration was observed at the twelfth donation, when the concentration decreased by 0.65g/dl to 6.84 +/- 0.47g/dl (\( P = 0.0001 \)).
The mean total IgG concentration for treatment horses was 6,666 +/- 2160mg/dl at the time of initial plasmapheresis (Table 3.1 and Figure 3.2). In comparison to this baseline concentration, a statistically significant decrease in IgG was detected at donations number five ($P = 0.02$), six ($P = 0.02$), seven ($P = 0.04$), ten ($P = 0.04$) and fourteen ($P = 0.03$).

**Discussion**

The automated plasmapheresis procedure itself was well tolerated by treatment horses. Despite the 2-6 hour-long procedure, only restlessness, stomping, mild anxiety and head shaking were observed. This is in agreement with results reported from three previous studies on automated equine plasmapheresis, which reported only mild restlessness occurring towards the end of the procedure (Feige K, Ehrat F, Kastner B, et al., 2003, Feige K, Ehrat F, Kastner B, et al., 2005, Magdesian K, Brook D, Wickler S, 1992). In contrast to the previous studies, mild sedation was administered to donors as needed during donation. Sedation allowed restless, anxious donors to relax and reduced the occurrence of stomping and head shaking.

Total plasma protein concentration in treatment horses dropped significantly by the third plasmapheresis donation and remained significantly decreased through the fourteenth donation. While the decline in total protein concentration was found to be statistically significant, the values were always well within the accepted reference interval of 6.0-8.5g/dl for total protein concentration in horses (Duncan J, Prasse K, 1986). Even more meaningful, the donors’ mean total protein concentration never decreased by two or more standard deviations in comparison to the baseline concentration. These findings suggest that 14-day intervals between donations provide
sufficient time for donors to replace lost proteins. This is in contrast to results of a study
that used automated plasmapheresis to remove a similar volume of plasma from first-time
equine donors (Feige K, Ehrat F, Kastner S, et al., 2005). Total protein concentration of
these donor horses decreased significantly and remained below the reference interval 21
days after a single procedure. Mean total protein concentration in the present study was
significantly decreased by the third plasmapheresis donation, however, the plasma protein
concentration did not continue to decline further with serial donations. Instead, it
remained at the same slightly decreased concentration through the fourteenth donation.
Perhaps the donors did not experience a continued decline because the frequent removal
of plasma proteins stimulated an increased synthesis and decreased catabolism of these
proteins, as suggested by Magdesian K, Brook D and Wickler S (1992). In addition,
donors may have become more efficient at shifting extravascular protein stores to the
intravascular space.

Mean IgG concentration in the treatment horses demonstrated an initial
statistically significant decrease by donation number five. In comparison to baseline, IgG
concentration dropped by 1,701mg/dl to a mean concentration of 4,965 +/- 2,075mg/dl.
A similar decrease in concentration was observed at donations number six, seven and ten,
with decreases of 1,782mg/dl, 1,523mg/dl and 1,619mg/dl, respectively. The largest
statistically significant decrease in IgG concentration occurred at donation number
fourteen, with a decrease of 2,403mg/dl. Despite significant decreases in IgG
concentration, donors did not demonstrate an increase in disease incidence after removal
of immunoglobulins. While decreases in IgG concentration were statistically significant,
the total IgG concentration never dropped below the accepted reference interval of 1,000-
1,500mg/dl for total IgG concentration in healthy adult horses (Tizard I, 2004). In fact, mean total IgG concentrations for the treatment horses remained well above the reference interval throughout the investigation. More notable, donors’ mean total IgG concentration never decreased by two or more standard deviations in comparison to the baseline concentration. It is reasonable to conclude that the discussed plasmapheresis program allows time for donor horses to replace lost IgG and does not put them at an increased risk for infectious disease development.

There was a tendency for baseline total protein and IgG concentrations of treatment horses to be increased in comparison to reference intervals established for healthy adult horses. A possible explanation for the increased baseline concentrations in the treatment horses is the repeated intradermal administration of project specific immunogen. The treatment horses received a minimum of six vaccinations with the immunogen prior to plasmapheresis donation number one. This suggests that vaccination with the immunogen may be responsible for the increased concentrations at baseline. Also, it is possible that populations of horses have naturally occurring higher IgG concentrations in comparison to established reference intervals. Observation of increased and highly variable IgG concentrations were identified in a short communication from de Camargo M, Kuribayashi J, Bombardieri C, et al. (2008), which used RID to determine the total IgG concentration of thirty-three horses aged between 6 and 23 years. Their study group had a mean total IgG concentration of 2,704 +/- 1424mg/dl, with 25% of their horses having concentrations greater than 4,000mg/dl.

In conclusion, performance of automated equine plasmapheresis in accordance with the described protocol did not result in sustained depletion of total plasma protein or
total IgG concentrations in the donors. This plasmapheresis program continued to harvest high-quality, IgG- and protein-rich plasma during the length of the investigation period.

**Footnotes**

a. Life Design Prime 14, Nutrena, Minneapolis, MN  
b. Quest Gel, Fort Dodge Animal Health, Fort Dodge, IA  
c. West Nile Innovator + EWT, Fort Dodge Animal Health, Fort Dodge, IA  
d. RabVac 3, Fort Dodge Animal Health, Fort Dodge, IA  
e. Fluvac Innovator EHV-4/1, Fort Dodge Animal Health, Fort Dodge, IA  
f. Pinnacle IN, Fort Dodge Animal Health, Fort Dodge, IA  
g. West Nile Innovator + EW, Fort Dodge Animal Health, Fort Dodge, IA  
h. Nolvasan Surgical Scrub, Fort Dodge Animal Health, Fort Dodge, IA  
i. ChloraPrep One-Step Antiseptic Sponge, Medi-Flex Hospital Products, Overland Park, KS  
j. Hospira, Inc., Lake Forest, IL  
k. BD, Franklin Lakes, NJ  
l. Supramid, S. Jackson, Inc., Alexandria, VA  
m. 4R-2252 Plasmacell-C, Baxter-Fenwal, Lake Zurich, IL  
n. Autopheresis-C A-200, Baxter-Fenwal, Lake Zurich, IL  
o. Baxter-Fenwal, Lake Zurich, IL  
p. EPS-20L Collection Bag, Charter Medical, Winston-Salem, NC  
q. Plasmalink Pooling Bottle, Baxter-Fenwal, Lake Zurich, IL  
r. Dormosedan, Pfizer Animal Health, Exton, PA
s. Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA

t. Plasma-Lyte A, Baxter-Fenwal, Lake Zurich, IL

u. Immuno-Tek Horse IgG ELISA, ZeptoMetrix Corporation, Buffalo, NY

v. EL808 Ultra Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT

w. KC Junior Microplate Data Analysis Software, Bio-Tek Instruments, Inc., Winooski, VT


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*Indicates a statistically significant ($p < 0.05$) change in concentration
Figure 3.1 Box plots for total plasma protein concentration of donors at each donation. 
+ indicates mean total plasma protein concentration 
* indicates a statistically significant (p < 0.05) different concentration in comparison to concentration at donation number 1
Figure 3.2  Box plots for total equine IgG concentration of donors at each donation.
+ indicates mean total equine IgG concentration
* indicates a statistically significant (p < 0.05) different concentration in comparison to concentration at donation number 1
CHAPTER IV

Conclusions

Development of an efficient, continuous-flow, automated plasmapheresis procedure to harvest equine plasma in accordance with current good manufacturing practices required patience and ingenuity. Human-model Baxter instruments and Plasmacell-C collection sets were modified for continuous-flow, dual-instrument operation. Adaptations were made in a manner that maintained sterility. A custom 20L-capacity bag was welded to modified sets to collect harvested plasma. Because the weight of product collected during equine plasmapheresis exceeded the capabilities of the instrument’s internal scale, an external scale was employed to weigh product.

Equine donors also presented unique challenges. Horses were placed in stocks during plasmapheresis, which allowed investigators to work safely with the horses during donation. To prevent excessive head and neck movement, all horses had their heads secured in padded slings and cross-ties occasionally were used. To assure sufficient blood flow to two plasmapheresis instruments simultaneously, a tourniquet was required to prevent collapse of the jugular vein. Because donors occasionally urinated or defecated during the procedure, surgical drapes were hung to protect the instruments and scales.

Despite challenges associated with development of an automated plasmapheresis procedure, a reliable method was achieved. The method described in this publication was used to perform more than 3,200 collections. Collection procedures typically lasted
between 3 to 6 hours and yielded approximately 20L of plasma per 900kg horse. Since 2005, 143 different horses participated in plasmapheresis and donated more than 50,000L of sterile plasma. Overall, the procedure was well tolerated by equine donors, which is in agreement with findings from previous studies that investigated equine plasmapheresis (Feige K, Ehrat F, Kastner S, et al., 2003, Feige K, Ehrat F, Kastner S, et al., 2005, Klages C, 2006, Magdesian K, Brook D, Wickler S, 1992). A majority of donors remained calm during the automated procedure and became more comfortable with plasmapheresis overtime.

Adverse events as a result of plasmapheresis were noted during the investigation. After repeated donations, one of the 143 donors developed marked and mildly painful scar tissue at the sites of catheterization. Fortunately, the scar formation never resulted in additional problems or inability to perform plasmapheresis. Inadvertent subcutaneous return of concentrated blood cells occurred during 0.7% of plasmapheresis procedures. This event was attributed to inaccurate catheter placement or catheter dislodgement and never resulted in further complications. Twenty-two plasmapheresis donations out of 3,240 were terminated early because donors exhibited increased respiratory rates, which continued to increase throughout the procedure. Neurological abnormalities were observed following 0.4% of procedures. Neurological signs were attributed to cerebral and cerebellar reperfusion injury. Modifications made to the tourniquet, tourniquet removal and post-plasmapheresis fluid administration eliminated occurrence of neurological problems. Three donor deaths related to the plasmapheresis procedure were documented during the investigation. The donors did not demonstrate abnormal clinical signs until after completion of the plasmapheresis procedure. Abnormal clinical signs
included tachycardia, tachypnea, pale mucous membranes and generalized weakness followed by collapse. Donor death occurred within 12 hours post-plasmapheresis. Aside from myocardial necrosis identified in one horse, necropsies performed on the donors were rather unremarkable and the definitive cause of death unknown. Overall, adverse events associated with the equine plasmapheresis procedure were minor, occurred with an extremely low frequency or were eliminated after making modifications. This is in agreement with results from studies that investigated adverse events associated with human plasmapheresis (Schulzki T, Seidel K, Storch H, et al., 2006, Tran-Mi B, Storch H, Seidel K, et al., 2004). The occurrence of adverse events related to human plasmapheresis was infrequent and included venipuncture complications, palpable hematomas and/or bleeding from venipuncture sites, citrate reactions, nausea and/or vomiting and dizziness. Despite the occurrence of adverse events related to the procedure, the investigators concluded that long-term intensive donor plasmapheresis was safe, provided that donors were carefully monitored.

The automated plasmapheresis procedure described in this publication was used to determine effects of serial plasmapheresis on total plasma protein and total IgG concentrations in equine donors throughout their involvement in a plasmapheresis program. This is the first time equine donors have been evaluated over an extended period of time while participating in such a program. Horses were approved for plasmapheresis once every 14 days and were permitted to donate a maximum 22ml of plasma per kg donor body weight. Donors experienced a significant ($p < 0.05$) decrease in both total protein and IgG concentrations during the investigation. However, mean total protein and mean total IgG concentrations never decreased by two or more standard
deviations from baseline. The decreases in concentration were not physiologically significant. Results from the investigation indicate that the plasmapheresis program does not cause sustained depletion of total protein or IgG concentrations. It would be interesting to evaluate a more intense and a less intense equine plasmapheresis program. Naïve or rested donors could be enrolled into programs with donations performed at 7 (more intense) and 28-day (less intense) intervals. It could be determined if plasma can be harvested from equine donors weekly without causing sustained hypoproteinemia. This would allow commercial operations to harvest and sell more product. Facilities with limited space and finances could use a smaller donor herd to meet production requirements. Or, collection of equine plasma at 28-day intervals could prove to be advantageous. The concentration of proteins and IgG in this plasma may be significantly greater than concentrations in plasma harvested from the more intensive programs.

The plasmapheresis technique described in this publication could be used to perform therapeutic equine plasmapheresis, a form of treatment never reported in equine medicine. Removal of pathogenic materials from circulation is the theoretical basis behind therapeutic plasmapheresis (Bartges J, 1997). Materials to be removed are proteins, protein-bound or high molecular-weight solutes and include: autoantibodies, circulating immune complexes, excess lipids or hormones, exogenous toxins and other abnormally occurring molecules. Therapeutic plasmapheresis has been used in human and canine medicine to manage autoimmune diseases, neoplasia, hyperviscosity, sepsis, intoxication and other conditions. The technique potentially could be used to help manage hyperlipemia in ponies and miniature horses. Plasmapheresis theoretically would hasten removal of excess triglycerides from blood. Plasmapheresis could treat
equine diseases that result from deposition of circulating immune complexes, including:
glomerulonephritis, vasculitis, purpura hemorrhagica or anterior uveitis.

The work described in this publication has several limitations. First, horses
selected for the investigation are not a randomly sampled group. Belgians, Percherons
and other draft breeds specifically were purchased from sales in the Midwest region of
the United States for inclusion in this study. Horses of different breeds and from
different geographical locations were underrepresented.

Second, an appropriate control group was not available for inclusion in the
investigation. It is possible that non-donor controls could have experienced similar
changes in total protein and IgG concentrations to that experienced in the donors.

Finally, eighteen treatment horses were included in the study evaluating the
effects of serial plasmapheresis. Treatment horses participated in a mean 13 consecutive
donations, with less than five horses participating in donations number 15-19. No
statistically significant changes were observed at donations number 15-19. The small
number of subjects associated with these donations could explain why the changes were
not considered statistically significant.
References


The AUUESPP
Operator’s Manual
for Equine
Plasmapheresis
# AUESPP Operator's Manual

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Section I

Introduction
Chapter 1: Introduction to the AUESPP
Introduction to the AUESPP

Welcome to Auburn University's Equine Source Plasma Project (AUESPP). The AUESPP operates on approximately 175 acres of secure pasture land located in north Auburn, Alabama. Over thirty individuals are employed as full- or part-time project members under the supervision of project director, Dr. Kenny Brock. Project employees include an international team of veterinarians, a quality control department, technical scientists, a project pharmacist, laboratory professionals and a crew of skilled farmhands. Maintained on the property is the project's impressive herd of nearly 200 draft horses. These horses are fundamental to the project's existence and continued success.

The AUESPP was established in October 2003 with the objective of harvesting equine source plasma. The AUESPP has developed a high-speed, high-volume, continuous-flow process of automated equine plasmapheresis. The designed closed system removes whole blood from donor horses, separates plasma from the cellular components of blood and simultaneously returns concentrated cells back to the donor horses. The procedure is accomplished through adaptations made to human model Baxter A-200 Autopheresis-C instruments and through sterile modifications made to 4R-2252 Plasmacell-C disposable sets. The AUESPP collects plasma from individual donor horses only after veterinary approval and a minimum fourteen day interval between plasmapheresis sessions. The designed method can safely harvest twenty liters of plasma from appropriate equine donors in less than six hours. Standard operating procedures (SOPs) have been developed, which detail the collection method to ensure that each liter of equine plasma is collected following current good manufacturing practices (cGMP). After collection, the plasma is sterilely aliquoted into appropriately labeled 1 liter high density polyethylene bottles and frozen at a set temperature of -35 degrees Celsius. The plasma is stored in a locked, alarmed and continuously monitored freezer. Designated lots of frozen plasma are packaged and shipped by the AUESPP upon request. The entire process is made possible only through the hard work of AUESPP's diverse and dedicated staff. Welcome!
Chapter 2: Introduction to the AUESPP Operator’s Manual
Introduction to the AUESPP Operator’s Manual

This AUESPP Operator’s Manual is designed as a guide for employees conducting equine plasmapheresis. This manual is to be used in conjunction with Baxter’s Operator’s Manual for the Autopheresis-C Plasmapheresis System. This AUESPP Operator’s Manual navigates employees through each step of the equine plasmapheresis procedure.

Manual goals:

- A. Introduce employees to the AUESPP
- B. Detail the modifications made to the Autopheresis-C instruments to allow for equine plasmapheresis
- C. Detail the modifications made to the Plasmacell-C sets to allow for equine plasmapheresis
- D. Describe the preparation required for an equine plasmapheresis procedure
- E. Describe how to perform an equine plasmapheresis procedure
- F. Provide a troubleshooting section that covers commonly encountered instrument messages and plasmapheresis events

Organization of the AUESPP Operator’s Manual

This manual is divided into four main sections:

1. **Section I** serves as an introduction.

2. **Section II** familiarizes employees with the instruments and disposable sets used during equine plasmapheresis at the AUESPP.

3. **Section III** contains information on how to properly prepare for equine plasmapheresis.

4. **Section IV** contains information on how to properly perform equine plasmapheresis.
Section II
The Instruments and Disposable Sets of Equine Plasmapheresis
Chapter 3:  
The Model A-200 Autopheresis-C Instrument
OVERVIEW

The purpose of this chapter is to introduce employees with the Model A-200 Autopheresis-C instrument and to describe modifications made to the instrument to allow for equine plasmapheresis at the AUESPP.

Chapter goals:

A. Become familiar with the Model A-200 Autopheresis-C instrument
B. Appreciate the modifications made to the instrument to allow for equine plasmapheresis

Model A-200 Autopheresis-C
AUTOPHERESIS-C INSTRUMENT COMPONENTS

Anticoagulant (AC) Pump
The pump delivers sodium citrate solution at a controlled rate into the blood line.

Blood Pump
The pump collects whole blood from donors and delivers anticoagulated blood to the separation device.

Cell Pump
The pump receives concentrated cells from the separation device and sends the concentrated cells through the cell line into the reservoir.

Clamps
The four clamps associated with the Autopheresis-C instrument open and close automatically during operation to control the flow of fluids traveling in the disposable set.

Hemoglobin Detector
The hemoglobin detector is an optical sensor that monitors the plasma line for red blood cells and/or hemoglobin during operation.

Venous Pressure (P1) Sensor
The P1 sensor connects to the disposable set and monitors the donor’s venous pressure during operation.

Transmembrane Pressure (P2) Sensor
The P2 sensor connects to the disposable set and monitors the transmembrane pressure within the separation device during operation.

Air Detector
The air detector is an ultrasonic sensor that detects air in the blood line.

Reservoir Monitor System
Four optical sensors monitor the fluid level in the reservoir- S1, RS1, S2, RS2.

Device Support
The support holds the separation device in place during plasmapheresis.

Motor Cup
The magnetic cup receives the separation device.

Pressure Cuff
The pressure cuff is wrapped around the donor’s vein and automatically inflates or deflates during operation.

Weigh Scale
The scale continuously weighs the plasma collected in the bag/bottle attached to the weigh scale hanger.
AUESPP Autopheresis-C Modifications:

1. The instrument Weigh Scale is not utilized during operation and the associated Weigh Scale Hanger is permanently removed from each instrument. Instead, an external Ohaus Champ Square CQ50L Scale with CD33 indicator is used for weighing purposes during equine plasmapheresis.

BEFORE

Hanger present

AFTER

Hanger removed

Ohaus Champ Scale with CD33 Indicator

2. The attached Pressure Cuff is not used on the equine donor during plasmapheresis. Instead, the instrument's Pressure Cuff is wrapped around a nearby portion of the stocks.
3. The Cell Pump functions to immediately return blood cells to the donor and not to the reservoir.

4. Switch #8 of the DIP Switch S1 is permanently placed in the ON position. This prevents the “CK Blood Flow” message from being repeatedly displayed throughout the procedure.

5. Plasmapheresis at the AUESPP only requires use of Chips U16 and U17 for instrument operation. Chips U14 and U15 are removed from the CPU Board of the Autopheresis-C instruments.

6. Autopheresis-C version APA 2.0 Software for Veterinary Applications is installed on the Model A-200 instruments.
7. The following Configuration/Default Matrix is set on each Autopheresis-C instrument:

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**NOTE:** A NO Saline Protocol is used at the AUESPP
Chapter 4: The Plasmacell-C Disposable Set
OVERVIEW

The purpose of this chapter is to introduce employees with the Baxter 4R-2252 Plasmacell-C set and to describe the method for creating the modified Plasmacell-C sets used in equine plasmapheresis at the AUESPP.

Chapter goals:

A. Become familiar with the Baxter 4R-2252 Plasmacell-C Disposable set
B. Appreciate the supplies and equipment required for modified Plasmacell-C set production
C. Understand how to build the modified Plasmacell-C set

Modified Baxter 4R-2252 Plasmacell-C Disposable Set
Plasmacell-C Components

**Donor Line**
This tubing line receives whole blood from the donor and transports it to the Autopheresis-C instrument.

**Anticoagulant Line**
This tubing line carries anticoagulant from its red spike, through the AC pump and delivers the sodium citrate to the whole blood at the AC Y.

**Blood Line**
This tubing line carries anticoagulated whole blood past the air detector, through the blood pump and to the separation device.

**Separation Device**
The separation device receives anticoagulated whole blood from the blood line. Plasma is separated from the cellular components of blood by a rotating membrane filter. The plasma exits the device and travels down the plasma line. The concentrated cells exit the device and are sent into the cell line.

**Plasma Line**
This tubing line receives plasma from the separation device. The plasma line passes through the hemoglobin detector and delivers plasma to the collection container.

**Cell Line**
This tubing line receives concentrated cells from the separation device, passes through the cell pump and delivers the cells to the reservoir.

**Reservoir**
The reservoir receives and stores concentrated cells from the cell line.

**Saline Line**
This tubing line begins at the saline Y and terminates in a clear spike.

**Venous (P1) Line**
This line connects to the venous pressure sensor which monitors the donor’s venous pressure during pheresis.

**Transmembrane (P2) Line**
This line connects to the transmembrane pressure sensor which monitors transmembrane pressure in the separation device during pheresis.
PLASMACELL-C: General Overview

Saline, P1 and P2 Lines

Separation Device

Cell Line

Reservoir

Plasma Line

AC, Donor and Blood Lines
PLASMACELL-C: Detailed Overview

AC Y
Donor Line
Blood Line
AC Line
Blood Line
AC Line Spike
Saline Line
Saline Line Spike
Blood Line
P1 and P2 Connectors
Cell Line
Plasma Line
Custom Tubing Sets and Collection Bag used in Modified Set Production

Charter Medical 03-220-EPS1 Tubing Set (EPS1)
This sterile custom tubing set consists of two 36inch (90cm) long lines which join at a Y junction. A single 76inch (195cm) long line extends from the Y piece. The single line contains an infusion port and ends in a luer connection. During equine plasmapheresis, this tubing carries concentrated cells from the cell lines of the modified disposable set to the horse's return catheter.

Charter Medical 03-220-EPS2 Tubing Set (EPS2)
This sterile custom tubing set consists of two 28inch (70cm) long lines which join at a Y junction. A single 36inch (90cm) long line with an infusion port extends from the Y piece. During equine plasmapheresis, this tubing carries plasma from the plasma lines to the EPS-20L collection bag.
Charter Medical 03-220-EPS3 Tubing Set (EPS3)
This sterile custom tubing set consists of two 36inch (90cm) long lines which join at a Y junction. A single 16inch (40cm) long line extends from the Y piece. The single line contains an infusion port and ends in a luer connection. During equine plasmapheresis, this tubing line attaches to the donor’s extraction catheter and delivers whole blood to the modified disposable set.

Charter Medical EPS-20L Collection Bag (EPS-20L)
This 20liter capacity gamma irradiated EVA bag is equipped with 3 ports. Two ports provide possible tubing connections, while the third port provides sterile sampling access. During equine plasmapheresis, plasma is delivered to the 20L bag through the centrally located tubing port.
Materials for Modified Plasmacell-C Set Production

Supplies

(2) Baxter 4R-2252 Plasmacell-C Disposable Sets
Charter Medical EPS1 tubing set
Charter Medical EPS2 tubing set
Charter Medical EPS3 tubing set
Charter Medical EPS-20L bag
36 inches (90cm) of spare sterile tubing
14 inch (30-35cm) saline line sealing guide
Nalgene tub
Bag liner
Lot # Tracking Card

Equipment

Terumo TSCD Sterile Tubing Welder
Baxter AutoSeal Tubing Sealer
Protocol for Building the Modified Plasmacell-C Set:

1. Open both 4R-2252 sets and place them on the work top surface. Orient the sets to position the separation device upright with the plasma line exiting the bottom of the device.

2. Locate the cell line of one 4R-2252 set. Using the Baxter AutoSeal Tubing Sealer, seal the cell line where it enters the reservoir. Repeat procedure on the remaining 4R-2252 set.

3. Locate the saline line of one 4R-2252 set. The saline line ends in a clear spike and is bundled with the P1 and P2 lines. Isolate the saline line and seal the line approximately 14 inches (30-35cm) from the spiked end using the sealing guide or a ruler. Separate the sealed line and discard. Repeat procedure on the remaining 4R-2252 set.
Protocol for Building the Modified Plasmacell-C Set:

4. Open the Charter Medical EPS3 tubing pack. Weld the donor line of one 4R-2252 set onto one 36inch (90cm) long line of the EPS3 tubing. Repeat procedure with the remaining 4R-2252 set and the remaining 36inch (90cm) long line of the EPS3 tubing. Bundle the EPS3 line to prevent tangling.

5. Open the Charter Medical EPS1 tubing pack. Separate the previously sealed cell line of one set and weld it onto one 36inch (90cm) long line of the EPS1 tubing. Repeat procedure with the remaining disposable set and the remaining 36inch (90cm) long line of the EPS1 tubing. Bundle the EPS1 line to prevent tangling.
Protocol for Building the Modified Plasmacell-C Set:

6. Locate the P2 line of one 4R-2252 disposable set. Lengthen the P2 line by welding approximately 18 inches (45-50 cm) of spare sterile tubing into the line. Weld the connector back onto the end of the lengthened P2 line. Repeat procedure on the P2 line of the remaining set.

NOTES:

- Following completion, the modified set is stored in a Nalgene tub
- The bag liner and Lot# Tracking Card are added to the tub
- Lot numbers and expiration dates for EPS1, EPS3 and Plasmacell-C Sets are identified on the Tracking Card
- Charter Medical EPS2 tubing and Charter Medical EPS-20L bag are added to the Nalgene tub and identified on the Tracking Card prior to initial set installation
- Charter Medical EPS2 tubing and Charter Medical EPS-20L bag are welded onto the modified disposable set during the “Install Set” portion of plasmapheresis. This welding procedure is described in detail in Chapter 6.
SUMMARY

The AUESPP Modified Plasmacell-C Set

Essentially, two separate 4R-2252 Plasmacell-C sets are welded together into one modified set for dual-instrument operation. The saline lines are sealed reflecting the NO SALINE PROTOCOL adopted by the AUESPP. The P2 lines are lengthened on the modified set to accommodate the frequently encountered elevated transmembrane pressures associated with equine plasmapheresis. Finally, the sets are modified to allow for the simultaneous processes of both collection and reinfusion. During equine plasmapheresis, the Charter Medical EPS3 tubing line receives whole blood from the donor’s extraction catheter. The EPS3 tubing carries whole blood to the donor lines of the modified set. Anticoagulant is added to the lines as the blood travels toward the two simultaneously operating Autopheresis-C instruments. The anticoagulated whole blood travels through the blood lines to each instrument’s separation device. Concentrated cells exit each separation device and enter the cell lines. During equine plasmapheresis, the concentrated cells bypass the reservoir and are immediately returned to the horse via the Charter Medical EPS1 tubing line. Plasma exits each separation device and travels down the plasma lines. During equine plasmapheresis, the plasma lines join the Charter Medical EPS2 tubing set, which carries the plasma to the EPS-20L collection bag.
Section III
Preparation for Equine Plasmapheresis
Chapter 5:
Preparing for Equine Plasmapheresis at the AUESPP
OVERVIEW

The purpose of this chapter is to describe the preparation required for an equine plasmapheresis procedure. Preparation is essential to a successful plasmapheresis session at the AUESPP. The collection facility must be organized, supplies should be inventoried and donors must be approved for collection.

Chapter goals:

A. Understand that preparation for plasmapheresis begins days in advance of the procedure
B. Appreciate the quantity of materials required to perform equine plasmapheresis
C. Become familiar with Collection Room organization
D. Understand how to perform initial installation of the modified Plasmacell-C set
E. Appreciate the veterinary preparation required for equine plasmapheresis

The Collection Room
Preparation for equine plasmapheresis at the AUESPP begins well in advance of the actual procedure. Specific preparatory tasks are carried out at different time intervals prior to an equine plasmapheresis session. The following is a detailed list of the tasks performed according to time.

I. Tasks Performed Greater Than 24 Hours Prior to Plasmapheresis: Supply Preparation

- Build modified Plasmacell-C sets for dual Autopheresis-C instrument operation
- Ensure there is a sufficient number of modified Plasmacell-C sets for plasma collection
- Order any necessary supplies from the Supplies Coordinator

### SUPPLY INVENTORY

#### General Supplies:

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4x4 gauze</td>
<td>1 inch/2.5cm white porous tape</td>
</tr>
<tr>
<td>Alcohol prep pads</td>
<td>Boxes of exam gloves sizes XS-XL</td>
</tr>
<tr>
<td>4 inch/10.2 cm Elastikon</td>
<td>4 inch/10.2 cm VetFlex cohesive bandage material</td>
</tr>
<tr>
<td>Infusion plugs</td>
<td>5 ml BD Vacutainer Plasma Preparation Tubes</td>
</tr>
<tr>
<td>Surgical drape material</td>
<td>BD Vacutainer Luer-Lok Access Devices</td>
</tr>
<tr>
<td>2-0 Supramid sutures</td>
<td>500 ml bags Baxter Anticoagulant Sodium Citrate Solution</td>
</tr>
<tr>
<td>#15 blades</td>
<td>1 ml, 3 ml, &amp; 30 ml syringes</td>
</tr>
<tr>
<td>Sandwich baggies</td>
<td>18 gauge &amp; 20 gauge 1.5 inch/3.8 cm needles</td>
</tr>
<tr>
<td>Adhesive/duct tape</td>
<td>5 L bags Baxter Plasma-Lyte A</td>
</tr>
<tr>
<td>Boxes of Kemwipes</td>
<td>72 inch/183 cm large animal IV sets</td>
</tr>
<tr>
<td>Sharps containers</td>
<td>Spray bottle of 70% ethanol</td>
</tr>
<tr>
<td>Detomidine</td>
<td>2% Lidocaine HCl</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>Labels</td>
</tr>
</tbody>
</table>

#### Scrub Cart Supplies:

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nolvasan Surgical Scrub</td>
<td>ChloraPrep One-Step applicators</td>
</tr>
<tr>
<td>4x4 gauze</td>
<td>Boxes of exam gloves sizes XS-XL</td>
</tr>
</tbody>
</table>
II. Tasks Performed 12-24 Hours Prior to Plasmapheresis: Collection Room Preparation

- Using adhesive, hang surgical drape material from the stocks
- Position two Autopheresis-C instruments adjacent to one another at the head of the stocks
- Wrap the Autopheresis-C instruments' blood pressure cuffs onto the stocks
- Plug the Autopheresis-C instruments into the yellow power cords
- Position one Ohaus Champ Square CQ50L Scale next to the Autopheresis-C instruments
- Balance the Ohaus Champ Square CQ50L Scale
- Hang two or three 500ml bags of Baxter Anticoagulant Sodium Citrate Solution from the solution pole of each Autopheresis-C instrument
- Place the following materials on the top of the Autopheresis-C instruments:
  - Several alcohol prep pads
  - Several infusion plugs
  - Several capped, sterile needles
  - One roll of 4inch/10.2cm Elastikon in a protective sandwich baggie
  - One roll of 4inch/10.2cm cohesive bandage material
  - Two 2-0 Supramid sutures
  - Two 3inch/7.6cm pieces of white porous tape
- Perform initial installation of the modified Plasmacell-C sets
- Open and place Charter Medical EPS2 tubing on top of Autopheresis-C instruments
- Open the EPS-20L bag, slip into the bag liner and place in the Nalgene tub
- Place the green Lot# Tracking Card on the work station cart
- Place a sharps container on the Collection Room floor next to Autopheresis-C instruments
- Place one tourniquet splint on the Collection Room floor next to the stocks
- Place the following West Nile Virus sampling supplies on the work station cart:
  - Two 5ml BD Vacutainer Plasma Preparation Tubes
  - One 3ml syringe with 20gauge 1.5inch/3.8cm needle attached
  - One BD Vacutainer Luer-Lok Access Device with 20gauge 1.5inch/3.8cm needle attached
- Place appropriate forms on each work station cart
- Place appropriate liners in medical waste and trash bins

*Temperature permitting
II. Tasks Performed 12-24 Hours Prior to Plasmapheresis: Collection Room Preparation

- Prepare the silver scrub cart with the following materials:
  - Canisters of 4x4 gauze
  - Squeeze bottles of Nolvasan Surgical Scrub
  - Boxes of exam gloves
  - Box of ChloraPrep One-Step applicators
- Draw up Sodium Citrate Solution into labeled 30ml syringes and place approximately 4-6 syringes on the Autopheresis-C instruments
- Draw up Detomidine into labeled 1ml syringes

The Prepared Collection Room
The Prepared Collection Room

Materials placed on the top of adjacent Autopheresis-C Instruments

Work station with medical waste and trash bins

WNV sampling supplies, paperwork and labeled syringes of Detomidine

Scrub cart
III. Tasks Performed Immediately Prior to Plasmapheresis: Final Preparation

- Turn on all Collection Room lights, exhaust fan and fly fan
- Plug Ohaus Champ Square Scales into the yellow power cords
- Perform the quality control check of the Ohaus Scales and record on the appropriate form
- Obtain the following from the Product Room:
  - Terumo TSCD Sterile Tubing Welders
  - Baxter AutoSeal Tubing Sealers
  - One cool pack per donor

The weights and paperwork used to QC the scales
Procedure for initial installation of the modified Plasmacell-C set:

1. Obtain from the Product Room a Nalgene tub containing a modified Plasmacell-C set, Charter Medical EPS2 tubing, Charter Medical EPS-20L bag, bag liner and Lot# Tracking Card.

2. Place the Nalgene tub and contents between the two adjacent Autopheresis-C instruments. The instruments remain OFF during this installation procedure.

3. Always begin disposable set installation on the Autopheresis-C instrument located nearest the head of the stocks.

4. Pick up one separation device and the tubing components associated with that separation device.

5. Insert the separation device into the motor cup and raise the device support upward. The separation device should click into place.

6. Stretch the sealed saline line across the top of the instrument and behind the saline clamp.

7. Slide the top of the reservoir into the reservoir channel. Press the bottom of the reservoir into the channel to lock into place. **NOTE:** Disposable set tubing should NOT be pinned behind the reservoir.

8. Identify the concentrated cell line where it exits the bottom of the separation device. Place the concentrated cell line into the tubing guide at the top of the cell pump. Roll the tubing onto the cell pump and into the tubing guide at the bottom of the pump. Close the cell pump by turning the handle.

9. Identify the blood line where it enters the separation device. Position the blood line around the cell pump, into the tubing guide and behind the blood clamp.

10. Position the reinfusion line behind the reinfusion clamp.

11. Slide the reservoir Y into the tubing guide above the blood pump.

12. Roll the blood line onto the blood pump and position the P1 Y against the tubing guide at the bottom of the blood pump. Close the blood pump.

13. Gently floss the blood line into the air detector. Spin the black wheel to a closed position.

14. Loop the venous pressure (P1) line around the blood pump and upward around the anticoagulant pump. **NOTE:** Position tubing into appropriate tubing guides.

15. Install the P1 line behind the left hand side of the pressure transducer cover. Pull the P1 line into both cover slots.
Procedure for initial installation of the modified Plasmacell-C set:

16. Install the transmembrane pressure (P2) line behind the right hand side of the pressure transducer cover. Pull the P2 line into both cover slots.

17. Approximately six inches (15cm) of P1 and P2 tubing should extend from the top of the pressure transducer cover. **NOTE:** Do not attach the connectors to the sensors and do not close the cover at this time.

18. Identify the anticoagulant (AC) tubing line. Loop the AC line onto the AC pump and close the pump door. The red tube stops should be positioned against the top and bottom tubing guides. Wrap the remaining AC line and associated red spike around the solution support pole until needed.

19. The plasma line is not manipulated at this time. Do not insert plasma line into the hemoglobin detector or place behind the plasma clamp.

20. Repeat steps 4 through 19 with the remaining disposable set and the remaining Autopheresis-C instrument.

21. Obtain three blue plastic hemostats from the work station cart. Place one hemostat on the Charter Medical EPS1 tubing line between the Y piece and the infusion port. Place the two remaining blue hemostats on each donor line before the AC Y.

22. Rest the Charter Medical EPS1 and EPS3 tubing lines between the adjacent instruments and onto the stocks.

**NOTES:**

- Check that none of the tubing lines are tangled
- Ensure that the tubing lines are correctly positioned onto the pump rollers
- Remember to place blue hemostats onto the tubing lines
- Following set installation:
  a. Open Charter Medical EPS2 and place on top of instrument
  b. Open Charter Medical EPS-20L bag, slip into bag liner and place in Nalgene tub
  c. Place Lot# Tracking Card on work station cart
- Modified Plasmacell-C set installation is completed during the plasmapheresis procedure
The Installed Plasmacell-C Set

- Sealed Saline Line
- P2 Line
- P1 Line
- AC Line
- Lengthened P2 Line
- Blood Line
- Separation Device
- Sealed Cell Line
- Reservoir
- Plasma Line
- Reinfusion Line
- Cell Line
- Blood Line
The Installed Plasmacell-C Set

- P1 and P2 tubing extending from transducer cover
- Separation device and reservoir properly installed
- One blue hemostat placed on each donor line
Veterinary Preparation Required for Equine Plasmapheresis

The veterinary preparation for equine plasmapheresis at the AUESPP begins approximately 24 hours prior to collection. The veterinary staff focuses on selecting a suitable donor and preparing the suitable donor for the procedure. The following describes the preparation performed by the veterinary staff.

I. Veterinary Tasks Performed within 24 Hours of Plasmapheresis

1. A donor horse is identified from the plasmapheresis schedule.

2. Appropriate paperwork for plasmapheresis is initiated.

3. A record review of the donor horse is conducted. There are two possible outcomes:
   i. The horse is not on deferral and determined suitable for plasmapheresis
   ii. The horse is on deferral and determined unsuitable for plasmapheresis

4. The suitable donor horse is brought to the Treatment Barn and examined.
   a. Horse’s identification is verified
   b. Physical exam is performed
   c. A large rectangle of hair is clipped from over each jugular vein
   d. EDTA (purple top) and Serum (red top) blood tubes are collected

5. The donor horse’s total protein concentration is determined using a refractometer. The total protein must be greater than 5.5g/dl.
II. Veterinary Tasks Performed Immediately Prior to Plasmapheresis

1. The suitable donor horse is brought to the Treatment Barn and its identity verified.

2. The horse is weighed on the Equine Platform Scale.

3. The horse’s temperature is determined using an NIST traceable rectal thermometer. The temperature must be less than 103.0°F/39.5°C.

4. The donor is:
   i. Accepted for plasmapheresis
   ii. Rejected for plasmapheresis
   iii. Temporarily deferred

5. The accepted donor horse is sedated with Detomidine intravenously and led into the Collection Room.

6. The horse is secured in the stocks with a rear rope and back rope. The horse’s head is situated in a padded sling, while its chest rests against the door of the stocks.

7. The clipped area over each jugular vein is prepared for catheterization using the supplies located on the silver scrub cart.
   a. Don exam gloves and obtain 4x4 gauze with a “clean” hand
   b. Transfer 4x4 gauze to “dirty” hand and squeeze Nolvasan Surgical Scrub onto gauze
   c. Scrub the entire clipped area with the gauze and Nolvasan Surgical Scrub
   d. Repeat process until the gauze remains clean following the scrub
   e. Obtain a ChloraPrep One-Step applicator with “clean” hand and release the antiseptic
   f. The applicator is rubbed over the jugular furrow with repeated strokes for 30 seconds
   g. Allow 30 seconds for the area to dry

8. Catheterization is performed by AUESPP veterinarians using the following method:
   a. Inject 2% Lidocaine HCl subcutaneously over each catheterization site
   b. Don sterile gloves
   c. Produce a small skin incision in each anesthetized area using a #15 blade
   d. Place a 10gauge 3inch/7.6cm BD Angiocath catheter directed ventrally into the horse's jugular vein
   e. Cap catheter with an infusion plug
   f. Place a 10gauge 3inch/7.6cm BD Angiocath catheter directed ventrally into the remaining jugular vein
   g. Cap catheter with an infusion plug
   h. Secure catheters into place with a suture
Veterinary Preparation

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><img src="image1" alt="Horse Scrubbed" /></td>
<td><img src="image2" alt="Horse Blocked" /></td>
<td><img src="image3" alt="Horse Catheterized" /></td>
</tr>
</tbody>
</table>

Veterinary Supply Inventory

- BD Vacutainer EDTA blood tubes (purple tops)
- BD Vacutainer Serum blood tubes (red tops)
- 20 gauge 1.5inch/3.8cm Vacutainer needles
- Capillary tubes and Clay
- Distilled water
- 1ml & 3ml syringes
- 18 gauge & 20 gauge 1.5 inch/3.8cm needles
- #15 blades
- Exam and Sterile Gloves
- 10 gauge 3 inch/7.6cm BD Angiocath catheters
- 2-0 Supramid sutures
- Infusion plugs
- 2% Lidocaine HCl
- Detomidine
- Butorphanol
Section IV
Performing Equine Plasmapheresis
Chapter 6:
Performing Equine Plasmapheresis at the AUDESPP
OVERVIEW

The purpose of this chapter is to describe the equine plasmapheresis procedure. It includes detailed information regarding instrument operation, donor health and record keeping.

Chapter goals:

A. Understand how to advance the Autopheresis-C instruments to proceed with plasmapheresis
B. Understand how to complete modified set installation
C. Understand how to prime the disposable set with anticoagulant
D. Grasp the concept of continuous-flow, equine plasmapheresis
E. Recognize the importance of monitoring and documenting the donor’s health
F. Know when and how to stop the equine plasmapheresis procedure
G. Recognize the importance of keeping accurate records

Equine Plasmapheresis
I. PLASMAPHERESIS: Instrument Start Up
The side-by-side Autopheresis-C instruments are powered on and advanced simultaneously.

1. Turn on Autopheresis-C instruments by flipping the power switch located on the back of the units.

2. “Self Test” appears on the display panel, followed by “Turn OFF Sw 8.” Press ▼ to advance.

3. “Test (Press STOP)” appears. Press the STOP button located on the far right of the operating panel. “Plasmapheresis” is displayed, press ▼ to advance.

4. Software information and protocol settings scroll across the display, followed by “NOT FOR HUMAN USE.” Press ▼ to advance.

5. Information regarding authorized use of the unit scrolls across the display, followed by “Calibration.” The Autopheresis-C verifies operating parameters during instrument calibration. “Install Set” is displayed after the instrument passes calibration.

II. PLASMAPHERESIS: Set Installation
Installation and modification of the dual Plasmacell-C set is completed during “Install Set.” A Terumo welder is required for the procedure.

1. Obtain the EPS2 tubing assigned to the modified set. Weld one 28 inch (70cm) long line of the EPS2 to one plasma line. Close the plasma line behind the hemoglobin detector door and slide the plasma line into the plasma clamp. Attach pressure sensors and close the transducer cover on the associated instrument.

2. Weld the remaining 28 inch (70cm) long line of the EPS2 to the remaining plasma line. Close the plasma line behind the hemoglobin detector door and slide the plasma line into the plasma clamp. Attach pressure sensors and close the transducer cover on the associated instrument.
3. Weld the 36inch (90cm) long line of the EPS2 to the centrally located tubing port on the designated EPS-20L bag. Place a cool pack on the bottom of the Nalgene tub and set the 20L bag with its liner on top.

4. Place the Nalgene tub and its contents onto the Ohaus Champ scale. Tare the displayed weight.

5. Continue with set installation by pressing the ▼ button. “Check Reservoir” is displayed. Verify that the reservoir is properly installed. Press ▼ to advance.

6. “CLAMP TUBING END” is displayed. Verify that blue hemostats are placed on each donor line and the EPS1 line. Press ▼ to advance.

7. “INSTALL CK” is displayed while the instrument performs an installation check. The Autopheresis-C instrument advances to “CONNECT AC” after a successful check.

III. PLASMAPHERESIS: Anticoagulant Priming
The entire EPS3 tubing set and both blood lines must be primed with sodium citrate solution before the Autopheresis-C instruments can be advanced to “Venipuncture.” Both solution connection and set priming are performed wearing exam gloves.

1. Don exam gloves and aseptically spike the sodium citrate bag of each instrument with the associated red AC line spike.

2. Grasp the EPS3 tubing set and move both blue hemostats from the donor lines to the blood lines. Remove the white cap from the luer end of the EPS3 line.

3. Solution priming is performed on each instrument individually. Prime the tubing by pressing ▼ on one instrument. “AC Priming” is displayed. The AC pump delivers sodium citrate at the level of the donor line. The sodium citrate continues down the EPS3 tubing. The blue hemostat is moved back to the donor line once the sodium citrate reaches the EPS3 Y. This allows the anticoagulant to prime the blood line. “Turn off dip switch 8” appears after the solution reaches the air detector. Priming of this instrument is complete.
4. Press ▼ to initiate priming on the remaining instrument. Sodium citrate is primed past the EPS3 Y and to the tip of the EPS3 luer when priming the second instrument. Then, the blue hemostat is moved back to the donor line to allow priming of the blood line. “Turn off dip switch 8” appears after priming is complete. Replace the white cap on the end of the EPS3 tubing.

**Note:** Both instruments will require two priming cycles. An alarm will sound and “NO FLUID DETECTED” will appear after the first cycle. Press the ▲ button to return to “Connect AC” display and press the ▼ button to repeat “AC Priming.”

5. Advance both instruments with the ▼ button. “Percent AC = 6%” is displayed. Continue to press ▼ until “OPEN BLOOD PUMP” appears. Open the blood pumps to equalize pressure throughout the modified set. Close the blood pumps and press ▼.

6. “Venipuncture” is displayed. Inflated the pressure cuffs to 36mmHg by pressing the + button.

### IV. PLASMAPHERESIS: Donor Verification and Connection

Donor verification, appropriate paperwork and disposable set connection to the donor must occur before the instruments are advanced further.

1. The donor’s primary and secondary identification is verified, along with the donor’s approval for plasmapheresis.

2. The accepted donor is assigned a unique collection number and appropriate plasmapheresis paperwork is initiated.

3. The unique collection number and affiliated bar code is labeled on the donor’s plasma preparation tubes and on the donor’s 20L collection bag. The collection bag is also labeled with the equine donor’s primary identification, secondary identification and the donor’s weight.

4. The two labeled plasma preparation tubes are used for donor West Nile Virus testing. The blood samples are obtained using the following method:
   - Don exam gloves
   - Swab infusion plug of catheter with alcohol pad
   - Remove trash blood from the jugular catheter using a 3ml syringe
   - Dispose of trash blood syringe in sharps container
   - Use access device to fill both plasma tubes
   - Tubes are gently inverted 8-10 times
   - The samples are spun in the IEC Centra centrifuge and frozen at -35 degrees C
West Nile Virus Sample Collection

1. Trash blood removed and access device inserted into catheter.

2. Labeled plasma tubes are filled and then inverted 8-10 times.

3. The samples are spun in the IEC centrifuge and later frozen.
5. EPS1 and EPS3 tubing lines are attached to the donor’s catheters via luer connections. EPS3 is routinely connected to the near/right catheter, while EPS1 is connected to the far/left catheter. Both tubing lines are secured to the donor’s neck with sutures.

6. A tourniquet is wrapped around the horse’s neck to provide adequate blood flow for plasmapheresis. The tourniquet consists of a roll of Elastikon, a roll of VetFlex bandage material and a PVC splint.

**Note:** Gloves are worn during sample collection, tubing-to-catheter connection, suture placement and tourniquet application.
V. PLASMAPHERESIS: Continuous-Flow Operation

The Autopheresis-C instruments are advanced to simultaneously perform collection and reinfusion cycles. During this portion of plasmapheresis, equine plasma is harvested in the 20L bag and concentrated blood cells are returned to the donor.

1. Remove the three blue hemostats present on the tubing lines of the disposable set. Use one hemostat to anchor the EPS1 and EPS3 lines next to the donor’s tourniquet. The remaining two hemostats are stored on the Autopheresis-C instruments.

2. Press ▼ on both Autopheresis-C instruments to initiate the priming sequence. The instruments perform a “BLOOD PRIME”, a “BLD PRIME B” and a “FILTER PRIME.” During the priming sequence, whole blood is extracted from the donor’s catheter, mixed with anticoagulant and used to prime the disposable set.

Whole blood is extracted from the donor’s catheter via the EPS3 tubing line. Anticoagulant is added to the whole blood as it reaches the blood line. During “BLD PRIME B,” anticoagulated blood is pumped into the bottom of the reservoir.
3. Following priming, the instruments begin collection and reinfusion. The blood flow and plasma flow is initially 60ml/min and 20ml/min, respectively.

4. The instruments automatically increase to a blood flow rate of 150ml/min. At the AUESPP, both instruments are routinely set to operate at 135ml/min by pressing the — button three times. Pressing the — or + button decreases or increases the blood flow rate by a value of 5ml.

5. During continuous-flow operation, one of three operating displays may be viewed. The displays are changed by pressing the F button. Press the F button one time to view blood flow, plasma flow, cuff pressure, venous pressure, transmembrane pressure and cycle number.
VI. PLASMAPHERESIS: Donor Records and Donor Health

The donor is closely monitored throughout the plasmapheresis procedure. The Autopheresis-C instruments and Ohaus scale are also closely observed for proper operation. Donor health and equipment status is documented during plasmapheresis.

1. Return to the donor’s records and document the start time of plasmapheresis. The start time is when the ▼ buttons are pressed to begin the priming sequence.

2. Determine the donor’s maximum allowable collection. At the AUESPP, 22ml of plasma is harvested for each kilogram of donor body weight. The specific gravity of plasma is approximately 1.035, which is used to convert volume into mass. The calculations are below:

<table>
<thead>
<tr>
<th>Maximum Allowable Volume</th>
<th>Donor Weight kg x 0.022L/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Allowable Weight</td>
<td>Maximum Allowable Volume L x 1.035kg/L</td>
</tr>
</tbody>
</table>

3. The equine donor is continuously monitored throughout the plasmapheresis procedure. Attention is paid to the donor’s behavior, vital signs and catheter placement. Donor pulse, respiratory rate, mucous membrane color and ECG are evaluated and recorded after harvesting approximately 10L of plasma and at the end of the procedure. These parameters may be monitored and recorded more frequently if necessary.

Observing the donor’s ECG

**Note:** The Autopheresis-C instruments are always located on the donor’s right side. This allows access for auscultation of the heart and for ECG attachment.
4. The donor is sedated during plasmapheresis under veterinary direction. The horse is sedated intravenously with Detomidine or Detomidine and Butorphanol. Intravenous sedation is administered through the infusion port on the EPS1 tubing line. The protocol is to don exam gloves, swab infusion port with alcohol pad and slowly push sedation into the line. The sedation accompanies the concentrated cells returned to the donor. Sedation administered during plasmapheresis is documented in the donor’s records.

5. Unexpected events related to donor health and unexpected alert messages related to equipment are documented on the records of plasmapheresis.

VII. PLASMAPHERESIS: Ending Plasma Collection

Once plasmapheresis is complete, records are finalized, the plasma is processed, the donor receives IV fluids and equipment is cleaned.

1. Plasmapheresis is terminated under the following conditions:
   - Upon achieving the maximum allowable collection
   - Donor’s health is in jeopardy
   - Autopheresis-C instruments fail to maintain plasma production
   - Facility power loss
   - Project Director’s request

2. The instruments are stopped by pressing the STOP button and the end time of plasmapheresis is documented.

3. The weight and calculated volume of plasma harvested is recorded. Remaining paperwork is completed.

4. EPS1, EPS3, AC and plasma lines are heat-sealed. The equine donor and plasma collection bag are separated from the disposable set. The disposable set is discarded in a labeled medical waste container.
5. The 20L collection bag and plasmapheresis records are transported for further processing.

![Scale, EPS-20L bag and collection records](image)

6. The tourniquet is removed from the donor’s neck and IV fluids are returned to the donor via gravity flow.

![Donors receiving post-plasmapheresis IV fluids](image)

**Note:** The AUESPP routinely administers 15L of Plasma-Lyte A.
7. Following fluid replacement, catheters and sutures are removed from the donor’s neck.

8. Excess blood is washed from the donor’s body and the horse is released to its designated pasture.

Pastured horses post-plasmapheresis
SUMMARY

Continuous-Flow Operation

1. Whole blood is removed from the donor horse via the EPS3 tubing line. This line divides and delivers blood to both donor lines of the modified Plasmacell-C set.

2. Whole blood enters the blood lines and is mixed with sodium citrate. The blood lines carry anticoagulated blood to each Autopheresis-C instrument.
3. Whole blood travels past the air detector, through the blood pump and up to the separation device. The separation device acts as a rotating membrane filter to separate plasma from the cellular components of blood.

4. Plasma exits the separation device, passes through a hemoglobin detector and continues down the plasma line.
5. The two plasma lines join together at the EPS2 Y piece. The EPS2 delivers the harvested plasma to the EPS-20L collection bag.
6. Simultaneously, concentrated blood cells exit the separation device and enter the cell line. The cells are pumped back to the donor through the EPS1 tubing line which is connected to the horse’s return catheter.
Chapter 7: Troubleshooting
OVERVIEW

The purpose of this chapter is to provide troubleshooting information to be used by employees during the plasmapheresis procedure.

Chapter goals:

A. Understand how to troubleshoot according to Autopheresis-C displayed messages

B. Understand how to troubleshoot according to conditions encountered during plasmapheresis
Troubleshooting according to a displayed message

**Air In Draw Line**

**Issue:** Air has entered the blood line during collection and is detected by the air sensor.

**Resolution:** Inspect luer connections and tubing welds for a possible leak. Ensure that the AC bag is properly spiked and that the bag is not empty. Press ▲ to resume plasmapheresis.

**Air…Push ` `-**

**Issue:** Air has entered the blood line during reinfusion/cycle reset and is detected by the air sensor.

**Resolution:** Press the — button allowing the instrument to enter an air purge. The display will change to “Purging Air” and the air is transported to the vented reservoir. Following the purge, the display reads “Check Air Then ↑.” Press ▲ to resume pheresis or press — to purge any remaining air.

**Air/Stop**

**Issue:** This is an inappropriate message that may occur during collection. The operating panel will not recognize employee responses.

**Resolution:** Remove instrument from service immediately and seek repair.

**Backup (-); Resume (+)**

**Issue:** This display flashes when ▲ is pressed during plasma collection.

**Resolution:** Press + to resume plasma collection.

**Blocked**

**Issue:** The instrument is unable to return reservoir contents due to an obstruction in the blood/donor line or due to an obstruction in the catheter. This message is only displayed when the donor enters a reinfusion cycle.

**Resolution:** This message should not occur during equine plasmapheresis. If the message does appear, be sure to remove the reservoir completely when manually resetting the instrument cycle. Replace the reservoir only after “Cuff” is displayed.
Issue: This is an inappropriate message that may occur during collection. The operating panel will not recognize employee responses.

Resolution: Remove instrument from service immediately and seek repair.

Check AC Bag = 500ml

Issue: The 500ml bag of sodium citrate solution is nearly empty and requires replacement.

Resolution: Place a blue hemostat on the AC line and remove the line while wearing exam gloves. Spike a new 500ml bag of sodium citrate and remove the blue hemostat. Press ▲ to resume plasmapheresis.

Check AC Side or Check PC Side

Issue: The disposable set is unable to pass the installation check.

Resolution: Inspect the modified Plasmacell-C set for proper installation. Ensure that all pumps are closed, P1 and P2 lines are attached to appropriate sensors, blue hemostats are placed in proper locations and all tubing welds are popped open. Press ▼ to return to “Install Set” display and continue with the installation check. If the instrument does not recognize the employee response, open and then close the blood pump. Try pressing ▼ again. If the instrument still fails to recognize the employee response, turn instrument off and then back on. Advance the instrument to the “Install Set” display and continue with the installation check. Identify the set as unsuitable if it cannot pass the installation check after numerous attempts and install a new modified set.

Check Blood Flow

Issue: The instrument has exceeded the whole blood processing limit for a single collection cycle. The message is repeatedly displayed throughout the remainder of the cycle. This event should not occur during automated equine plasmapheresis at the AUESPP.

Resolution: Ensure that switch #8 if the DIP Switch S1 is placed in the “on” position.
Check M# Dir

**Issue:** An instrument pump is not operating properly.

**Resolution:** Resume plasmapheresis by pressing the ▲ button. It is not uncommon for this message to appear multiple times during the remainder of the procedure. Attempt to use the instrument to complete pheresis and remove from service for repair following the procedure.

Check P1

**Issue:** #1 The transducer cover plate is open.

**Resolution:** #1 Close transducer cover plate and press ▲ to resume.

**Issue:** #2 The blood level in the P1 line has reached the optical sensor indicating an increase in donor venous pressure.

**Resolution:** #2 Inspect the donor and disposable set for a cause of the pressure increase. Inspect the P1 sensor for blood and clean if necessary. Tighten the P1 filter connector onto the P1 sensor. If necessary, decrease the size of the P1 tubing loop or lengthen the P1 line. Press ▲ to resume.

Check P2

**Issue:** #1 The transducer cover plate is open.

**Resolution:** #1 Close transducer cover plate and press ▲ to resume.

**Issue:** #2 The blood level in the P2 line has reached the optical sensor indicating an increase in transmembrane pressure.

**Resolution:** #2 Inspect the donor and disposable set for a cause of the pressure increase. Inspect the P2 sensor for blood and clean if necessary. Tighten the P2 filter connector onto the P2 sensor. If necessary, decrease the size of the P2 tubing loop or lengthen the P2 line. Press ▲ to resume.

Check Venipuncture

**Issue:** An inadequate volume of whole blood is reaching the Autopheresis-C instrument.

**Resolution:** Stop both instruments to inspect the donor’s catheter and tubing lines. Ensure that the horse’s extraction catheter is still within the jugular vein. Flush the catheter with sodium citrate if blood flow is not visible. Inspect the EPS3 line for clot formation and flush any clots free with sodium citrate. Look for other possible obstructions in the tubing lines (kinks, blue hemostats, a donor leaning across tubing lines). Resume plasmapheresis with ▲.
Cuff Stabilizing

Issue: The blood pressure cuff is attempting to maintain the set pressure.

Resolution: Ensure that the cuff is properly secured around the stocks. Inspect the cuff and the cuff line for any leaks. Check that the cuff line is attached to the port located on the instrument. Press ▼ to advance with the plasmapheresis procedure.

HB Detect

Issue: The hemoglobin detector senses whole red blood cells, free hemoglobin, excessive lipids or excessive air bubbles in the plasma line.

Resolution: Inspect the plasma line for the presence of red blood cells, hemoglobin, lipids or air bubbles. Resume plasmapheresis with the ▲ button and continue to examine the plasma line. Air bubbles should eventually clear from the line. The presence of lipids during equine plasmapheresis is not a regular issue. If red blood cells or free hemoglobin continue to pass through the plasma line, the “HB Detect” alarm will sound again. This indicates that the separation device is allowing red blood cells and/or hemoglobin to pass into the plasma line. This set requires replacement.

High TMP/Check AC

Issue: An elevated transmembrane pressure is detected in the separation device.

Resolution: Inspect the plasma line, the concentrated cell line, the EPS2 tubing and the EPS1 tubing for an obstruction. Verify that the AC bag is properly spiked and that the bag is not empty. Resume plasmapheresis.

Install Set Standby

Issue: This message is displayed after the instrument has set idle during “Install Set.”

Resolution: Press ▼ to return to “Install Set.”

No Fluid Detected

Issue: Anticoagulant did not reach the air detector following the AC priming cycle.

Resolution: Verify the blood line is installed in the air detector. Verify that the AC bag is properly spiked. Press the ▲ button to return to “Connect AC” display and press the ▼ button to repeat “AC Priming.”
Overcurrent

**Issue:** A pump is drawing more current than expected.

**Resolution:** Examine all 3 pumps and inspect the tubing installed on the pumps. If no damage is detected, restart instrument by pressing the ▲ button. If the message appears repeatedly throughout the remainder of the procedure, remove the instrument from service for repair.

Overspeed

**Issue:** An instrument pump is not operating properly.

**Resolution:** Resume plasmapheresis by pressing the ▲ button. It is not uncommon for this message to appear multiple times during the remainder of the procedure. Attempt to use the instrument to complete pheresis and remove from service for repair following the procedure.

P Range Error

**Issue:** Instrument pressure sensors are registering inaccurate readings during calibration.

**Resolution:** Examine transducer ports for possible blockage and remove prematurely placed P1 or P2 connectors. Turn instrument off and restart.

P1/P2/P3 Sensor Error

**Issue:** Instrument pressure sensors are registering inaccurate readings during calibration.

**Resolution:** Examine transducer ports for possible blockage and remove prematurely placed P1 or P2 connectors. Examine the pressure cuff for any potential problems. Turn instrument off and restart.

Plug In Power Cord

**Issue:** The primary AC power supply to the Autopheresis-C instrument is interrupted.

**Resolution:** Assure that the power cord is plugged into both the power cord receptacle located on the instrument column and into the electrical outlet. If the issue does not resolve, there may be an internal AC power failure. In this situation, remove the instrument from service and seek repair.
Return (↓) Resume (↑)

**Issue:** This display flashes when ▼ is pressed during plasma collection.

**Resolution:** Press ▲ to resume plasma collection. Press ▼ if a cycle reset is desired.

-Slope

**Issue:** This message indicates an erroneous pressure reading.

**Resolution:** Press ▲ to resume plasmapheresis.

VarErr: #

**Issue:** This is an inappropriate message that may occur during collection. The operating panel will not recognize employee responses.

**Resolution:** Remove instrument from service immediately and seek repair.

24 Volt Relay

**Issue:** This message indicates a problem with the driver board.

**Resolution:** Remove instrument from service and seek repair.
Section IV  Chapter 7

Troubleshooting according to encountered conditions

**Condition:** Dropping Blood Flow Rates

**Description:** The blood flow rate is dropping from the set value for operation. This drop occurs on one instrument or on both instruments. This drop is the result of one or multiple variables. Several resolution options are available in this event. It is likely that multiple resolutions are employed.

**Resolution 1:** Examine tourniquet for proper placement and tension. Replace if necessary.

**Resolution 2:** Examine EPS3 and blood lines for kinks.

**Resolution 3:** Reset the instrument cycle. This is the best resolution if the rate is dropping for the initial time during the procedure.

**Cycle Reset Procedure A:**

1. Press ▼ twice
2. Remove reservoir from channel
3. Instrument display reads “RETURN” and then “CUFF”
4. Replace reservoir after “CUFF” appears
5. Instrument performs “BLD PRIME B”
6. Gently squeeze the blood line during “BLD PRIME B,” the venous pressure drops
7. Instrument performs “FILTER PRIME”
8. Instrument operates at 60/20 calibration mode
9. Instrument returns to continuous-flow operation

**Cycle Reset Procedure B:**

1. Remove reservoir from channel
2. Occlude reservoir sensor RS2 located at the very top of the channel
3. “Reservoir Overflow” message is displayed
4. Press ▲ button, “…WAIT” is displayed
5. “RETURN” and then “CUFF” is displayed
6. Replace reservoir after “CUFF” appears
7. Instrument performs “BLD PRIME B”
8. Gently squeeze the blood line during “BLD PRIME B,” the venous pressure drops
9. Instrument performs “FILTER PRIME”
10. Instrument operates at 60/20 calibration mode
11. Instrument returns to continuous-flow operation

**Resolution 4:** Stop Autopheresis-C instruments and examine the extraction catheter.

**Procedure:**

- Don exam gloves
- Close EPS3 line with a blue hemostat placed between the infusion port and the Y piece
- Disconnect EPS3 luer from extraction catheter
• Evaluate catheter for blood flow and verify that the catheter is in the vein
• Flush catheter with sodium citrate solution to remove any clots
• Reconnect tubing, remove hemostat, remove exam gloves and press ▲ to resume plasmapheresis.

Resolution 5: Stop Autopheresis-C instruments and examine the EPS3 luer for fibrin clot formation.
Procedure:
• Don exam gloves
• Close EPS3 line with a blue hemostat placed between the infusion port and the Y piece
• Disconnect EPS3 luer from extraction catheter and cap catheter with infusion plug
• Swab EPS3 infusion port with alcohol pad
• Use 30ml syringes of sodium citrate solution to flush out the luer of the EPS3 tubing
• Remove fibrin clots
• Reconnect tubing, remove hemostat, remove exam gloves and press ▲ to resume plasmapheresis

Resolution 6: Stop Autopheresis-C instruments and flush EPS3/blood lines for clots.
Procedure:
• Don exam gloves
• Close EPS3 line with a blue hemostat placed between the infusion port and luer
• Swab infusion port of EPS3 with alcohol pad
• Close blood line of one Autopheresis-C instrument with a blue hemostat and press ▲ to resume plasmapheresis on the opposite instrument
• Send 30ml syringes of sodium citrate solution through the EPS3 infusion port to the operating instrument
• Flush free any clots in the EPS3 and blood lines
• Repeat procedure on remaining instrument
• Remove hemostats and resume pheresis
**Condition:** Single Instrument “HB Detect”

**Description:** Hemoglobin and/or red blood cells are passing through the separation device into the plasma line. The “HB Detect” alarm sounds, the set is inspected and plasmapheresis resumes by pressing ▲. The plasma line does not clear and the “HB Detect” alarm sounds again. Half of the modified disposable set requires replacement.

**Resolution:** Replace half of the modified set. The remaining set and instrument may operate during the replacement procedure.

Single Set Replacement Procedure:

1. Seal the plasma line just below the plasma clamp.
2. Seal the cell line where it exits the cell pump.
3. Seal the donor line next to the AC Y.
4. Seal the AC line distal to the red spike.
5. Turn instrument off and remove trashed set.
6. Turn instrument on and advance to “Install Set.”
7. Obtain new Plasmacell-C set and install onto instrument.
8. Seal saline and cell lines.
9. Place a blue hemostat on the remaining plasma/EPS2 line. Weld the remaining plasma/EPS2 line to the plasma line of the new set.
10. Place a blue hemostat on the remaining cell/EPS1 line. Weld the remaining cell/EPS1 line to the cell line of the new set.
11. Place a blue hemostat on the donor line of the new set. Weld the remaining donor line to the clamped donor line of the new set.
12. Lengthen the P2 line.
13. Complete set installation and advance the instrument to “INSTALL CK.”
14. “CONNECT AC” is displayed. Don gloves and connect AC bag with red spike.
15. Press ▼ to prime blood line. **Note:** Only one priming cycle is necessary and blue hemostats are not manipulated.
16. Advance set to “Venipuncture” and inflate cuff to 36mmHg.
17. Stop the opposite instrument, remove blue hemostats and press ▼ to begin the priming sequence on the new set.
18. Complete appropriate paperwork.
Condition: Dual Instrument “HB Detect”

Description: Hemoglobin and/or red blood cells are passing through both separation devices into the plasma lines. The “HB Detect” alarm sounds, the dual set is inspected and plasmapheresis resumes by pressing ▲. The plasma lines do not clear and the “HB Detect” alarm sounds again. The modified disposable set requires replacement.

Resolution: Replace modified set.

Procedure:
- Seal the EPS1, EPS3, AC and plasma lines of the trashed set.
- Turn off the instruments and remove set.
- Turn instruments on and advance to “Install Set.”
- Install the new modified set.
- Weld the plasma lines to the remaining EPS2 which is already welded to the partially filled EPS-20L collection bag.
- Advance the instrument through “Install Set” and remaining prompts as normal.
- Complete appropriate paperwork.

Condition: Pressed ▲ at “Venipuncture” instead of ▼.

Description: The ▼ buttons are pressed to advance from “Venipuncture” to the priming sequence. If the ▲ buttons are pressed, the instruments will return to “Open Blood Pump.”

Resolution: Replace all three hemostats back onto the EPS1 and donor lines. Open the blood pump and then close the blood pump. Advance the instruments to “Venipuncture” and remove the three blue hemostats. Verify that the pressure cuffs are inflated and continue with the priming sequence by pressing the ▼ buttons.