TRANSFUSION FOR THE MILITARY WORKING DOG

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These clinical practice guidelines focus on transfusion for military working dogs injured as the result of trauma in a forward deployed environment and will focus only on canine blood products expected to be found in theater.

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INTRODUCTION

Military Working Dogs (MWDs) are at the same risk for injury as their human counterparts and when they are severely injured, best practice for resuscitation mirrors those recommended for humans.¹ A recent study of trauma in MWDs shows that explosions, gunshot wounds and lacerations account for the vast majorities of injuries sustained within the Central Command area of responsibility.² In severe cases, these mechanisms of injuries could require resuscitation with blood products.^{3,4} Hemostatic resuscitation has been shown in a many species to be superior to resuscitation with crystalloids or synthetic colloids^{5,6} and should therefore be considered the first-choice resuscitation product in severely wounded MWDs.

Note: only canine blood products should be considered for transfusion to an MWD as transfusion of human blood products into canines carries a high likelihood of an incompatible hemolytic reaction.⁷

INDICATIONS

Transfusion therapy is indicated for MWDs experiencing any condition (trauma, neoplasia, other) that results in acute blood loss or clinical anemia, or that significantly increases their bleeding risk (e.g., severe thrombocytopenia, Acute Traumatic Coagulopathy/Trauma-Induced Coagulopathy,⁸ other). Consider transfusion therapy as a first-line intervention for perioperative hemodynamic optimization in MWDs that have suffered major trauma. These guidelines focus on transfusion for MWDs injured as the result of trauma in a forward deployed environment and will focus only on canine blood products expected to be found in theater – specifically canine Whole Blood, Fresh Frozen Plasma and Freeze Dried Plasma. These guidelines should therefore not be used for all conditions.

Table 1. Definitions

Definitions
 Acute Blood Loss/Hemorrhage indicated if one or more of the following: Estimated or anticipated blood loss > 20% of estimated total blood volume (eTBV), ~ 400 - 500 mL in a 25 kg MWD based on eTBV of 85 mL/kg in MWDs. Systemic signs of hypoperfusion (See Table 6.) SBP < 90 mm Hg or drop in SBP by ≥ 30 mm Hg Shock index (SI) > 1.0 - (Heart rate/systolic blood pressure)⁹ Hypothermia (< 99°F or < 37°C) Hyperlactatemia (≥ 5 mmoL/L) and / or Base Excess -6.6 or lower
 Clinical Anemia resulting in: Tachycardia (HR ≥ 160 ± 20 beats/minute) ± bounding femoral pulses Pale to white mucous membranes Depressed mentation Hematocrit <20%, Hemoglobin < 7 g/dL Note: the above values are not transfusion triggers in dogs. Clinicians are encouraged to use multiple clinical and laboratory values to determine the need for transfusion in an anemic dog. Presence of worsening anemia with signs of impaired oxygen delivery: Hypoxemia (SpO2 < 90%, room air) Elevated RR (> 40 breaths/min) with increasing respiratory effort

Definitions

Clinical Coagulopathy

- Clinical signs of spontaneous hemorrhage with one of the following:
 - Viscoelastic tracing (thromboelastography and rotational thromboelastometry [TEG / ROTEM]) demonstrating decreased clot strength of < 40% of mean reference value [(ROTEM - Maximum Clot Firmness (MCL) or TEG - Maximal Amplitude (MA)]
 - Prolonged coagulation assays (activated Partial Thromboplastin Time [aPTT]; Prothrombin Time, [PT]) > 1.5 times the mean laboratory reference interval.

Standard transfusion triggers are generally not applied to canine medicine but rather the clinical condition of the MWD is assessed to determine the appropriateness of a transfusion. The following should be considered in the assessment for blood transfusion needs in a MWD:

Table 2. Mechanism of injury, anatomical site and severity of injury

Mechanism of injury, anatomical site and severity of injury

- 1. Mechanism of injury (MOI)
 - a. Blunt* Vehicular, primary blast injury.
 - b. Penetrating*
 - Stabbing, ballistic, impalements, bite wounds, secondary blast injury.
 - Higher-velocity, higher-kinetic-energy vs. low-velocity, lower kinetic
 - c. Other: Acceleration/deceleration; shear; and crush.

*Major trauma from blunt or penetrating MOIs present the greatest risk for massive hemorrhage warranting transfusion needs.³

2. Anatomical location: Internal versus External

- a. Cavitary bleeding (thoracic, peritoneal, retroperitoneal) is considered a major source of hemorrhage in trauma patients.
- b. Canines suffering blunt trauma to the abdomen have a higher risk for mortality due to unrecognized internal bleeding.¹⁰

3. Severity of Trauma

- a. Polytrauma
- b. Modified Animal Trauma Triage (ATT) score (see Table 4). ^{10,11}

Used collectively, the MOI (e.g., blunt force from primary blast wave), anatomical injury patterns (e.g., thoracic versus abdominal), external physical examination findings (e.g., bruising around the umbilicus, distended abdomen with positive fluid wave test, localized pain or tenderness, etc.) and accompanying vital parameters may increase the index of suspicion and direct attention to the most likely sources of occult hemorrhage.



Table 3. Physical parameters and laboratory indicators to evaluate the presence of hemorrhagic circulatoryshock warranting hemostatic resuscitation

	Physical parameters and laboratory indicators to evaluate the presence of hemorrhagic circulatory shock warranting hemostatic resuscitation				
1.	 Physical: a. Mentation b. Heart rate (HR) and rhythm, Capillary Refill Time (CRT) and mucous membrane color (MM) c. Femoral pulse rate and quality d. Rectal temperature e. Respiratory rate (RR) and respiratory effort 				
2.	External Injury patterns Bruising around the umbilicus, distended abdomen with positive fluid wave test, localized pain or tenderness, other.				
3.	 Laboratory and Diagnostic findings a. Packed cell volume (PCV) and Total solids (TS) b. Lactate and Base Excess (BE),¹² venous or arterial c. Pulse oximetry (SpO2) d. Systolic Blood Pressure (SBP) e. Shock index (SI) 				
4.	Coagulation: a. Coagulation assays (aPTT; PT) b. Viscoelastic tests (e.g. TEG [®] , ROTEM [®] , Sonoclot [®]) c. Platelet counts d. Fibrinogen				
5.	 Diagnostic imaging a. Survey radiographs b. Focused Assessment with Sonography for Trauma (FAST)¹³ Abdominal FAST (AFAST) with abdominal fluid score (AFS) Thoracic FAST (TFAST) – Pericardial site and Diaphragmatic-hepatic site 				
6.	Other Abdominocentesis or Diagnostic Peritoneal Lavage (DPL) with body cavity fluid analysis.				

as the MWD casualty is transported to higher roles of medical care.



Table 4. Modified Animal Trauma Triage (ATT) Score

Modified ATT Score ¹¹			
Perfusion			
MM pink/moist, CRT 2 sec, T \geq 100F, strong or bounding femoral pulse quality	0		
MM hyperemic or pale pink, MM tacky, T ≥ 100F, CRT 0–2 sec, fair femoral pulses	1		
MM very pale pink & tacky, CRT 2–3 sec, T < 100F, detectable but poor pulses	2		
MM gray/blue/white, CRT > 3 sec, T < 100F, non-palpable femoral pulses	3		
Respiratory			
Regular respiratory rate with no stridor, no abdominal component to respiration	0		
Mild increased respiratory rate and effusion, ± abdominal comp, mild upper airway sounds	1		
Mod increased respiratory rate and effort, some abdominal component, elbow abduct, mod increased upper airway			
Marked respiratory effort or gasping/agonal respiratory, little/no air passage	3		
Neurologic			
Central: consciousness: alert to slightly dull, interest in surrounding. Peripheral: normal spinal reflexes; purposeful movement and nociception in all limbs.	0		
Central: dull/depressed/withdrawn. Peripheral: abnormal spinal reflexes with purposeful movement and nociception intact in all 4 limbs.	1		
Central: unconscious, responds to noxious stimuli. Peripheral: absent purposeful movement with intact nociception in 2 or more limbs or nociception absent in 1 limb, decreased anal or tail tone.			
Central: nonresponsive to all stimuli, refractory seizures. Peripheral: absent nociception in 2 or more limbs, absent tail or perianal nociception.	3		
*Score 0–3 (3 = most severe) for each of the above 3 categories. Increased ATT score equates to more s injury.	severe		

Table 5: Diagnostic Recommendations

Diag	Diagnostic Recommendations					
•	Obtain serial (not a one-time measure) PCV and total solids to help assess the degree of ongoing hemorrhage. It is important to interpret the PCV in light of the TS concentration, and the acuteness of the blood loss.					
•	Incorporate serial measurements of base excess and lactate in conjunction with traditional clinical perfusion parameters (e.g., heart rate, pulse quality, mucous membranes, capillary refill time, mentation, body temperature, and arterial blood pressure) as end points of perfusion.					
•	Monitor for changes in vital signs and laboratory parameters that indicate progressing hypoperfusion such as increasing heart or peripheral arterial pulse rate with decreasing peripheral pulse strength, pale MM, prolonging CRT, and worsening anemia and lactate /BE values.					
•	Utilize FAST to evaluate the abdominal cavity, retroperitoneal space, pleural space, gastrointestinal tract, and fascial planes around fractured hopes for areas of "hidden" opgoing hemorrhage that may					

 Othize FAST to evaluate the abdominal cavity, retropentoneal space, pieural space, gastrointestinal tract, and fascial planes around fractured bones for areas of "hidden" ongoing hemorrhage that may lead to life-threatening hemorrhagic shock.



- Conduct serial assessments to evaluate whether cavitary effusion is developing or worsening over time.
- Use viscoelastic tests, when available, to identify the presence of a coagulopathy, predict transfusion needs, and evaluate the response to therapy.
- When viscoelastic tests are not available, transfuse blood products as needed to maintain PT and aPTT <1.5× of mean reference values.
- Consider ongoing retroperitoneal hemorrhage for any blunt abdominal trauma patient with refractory hemorrhagic shock and declining PCV/TP values, but no significant findings on abdominocentesis, DPL, or AFAST.

FAST - Focused Assessment with Sonography for Trauma; **PT** – Prothrombin Time; **aPTT** – activated Partial Thromboplastin Time; **DPL** – Diagnostic peritoneal lavage;

Table 6: Clinical and diagnostic considerations for predicting transfusion needs

Clinica	Clinical and Diagnostic Considerations for Predicting Transfusion Needs			
Gener	General			
	MWDs that present with overt hypotension or a high shock index consider early institution of hemostatic resuscitation (transfusion therapy is preferred to crystalloid as the initial resuscitation fluid for management of hemorrhagic shock).			
•	 Common clinical manifestations in canine trauma patients warranting blood transfusions, include³: Tachycardia (160 ± 20 beats/pulses per minute) Tachypnea (> 40 breaths per minute) with increased respiratory effort. Hypothermia (< 99°F or < 37°C) Hyperlactatemia (≥ 5 mmoL/L) 			
	In face of major blunt or penetrating trauma, a SI > 1.0 is a sensitive and specific adjunct tool for detecting acute small volume blood loss in dogs. It serves as a more valuable diagnostic assessment for detecting acute occult blood loss, as compared to assessing HR and SBP alone. Consider this a useful tool for the early identification of MWDs that are at risk for ongoing blood loss and decompensatory shock. ⁹			
•	Canines experiencing major trauma may present or become hypocoagulable . These trauma- associated coagulopathies are positively correlated with the incidence of hemorrhage and transfusion requirements. ¹⁴			



nica	l and Diagnostic Considerations for Predicting Transfusion Needs
•	Highly consider blood transfusion in MWDs experiencing major trauma that have evidence of shock or worsening anemia and /or as a requirement for perioperative hemodynamic optimization . ³
	 Clinical signs indicative of hemorrhagic shock vary greatly and are not always readily recognizable; particularly, in the presence of intracavitary hemorrhage (pleural, peritoneal, or retroperitoneal).
	• Early identification of internal hemorrhage remains a challenge without the use of advanced imaging modalities (e.g., focused assessment with sonography for trauma, computed tomography). Casualties with severe internal hemorrhage may succumb to hypovolemic shock before a medical provider realizes the presence and extent of internal bleeding.
	• A hands-on physical examination has a low sensitivity for identifying internal bleeding, particularly, in patients suffering only blunt trauma with no external injuries; however, despite the absence of external injuries, internal hemorrhage is likely in any casualty suffering major blunt force or penetrating trauma with accompanying signs of circulatory shock (i.e. tachycardia, pale mucous membranes, prolonged capillary refill time, poor femoral artery pulse quality, depressed mentation, cold extremities).
	• Anatomical injury patterns (thoracic and abdominal trauma) with supportive physical examination findings (e.g. bruising around the umbilicus, distended abdomen with positive fluic wave test, localized pain or tenderness, etc.) may also direct the provider's attention to the most likely areas to explore for sources of internal hemorrhage.
•	Remain cognizant of occult hypoperfusion (cryptogenic shock) from ongoing hemorrhage,
	 particularly, in MWDs with blunt abdominal trauma. Although, overt hypotension is the most widely recognized sign of circulatory shock, it is also
	 indicative of more advanced shock. Traditional vital signs (HR and blood pressure) may not always serve as reliable markers of blood loss and decreased perfusion due to rapid activation of compensatory mechanisms.
V ar	nd TS interpretations
•	Serum PCV / TS are specific but insensitive predictors of subsequent transfusion in dogs.
•	Trauma patients with severe, acute hemorrhage may require transfusions at much higher PCVs that patients with more chronic disease or less systemic compromise. ¹²
•	In acute hemorrhage, PCV and TS values initially remain within reference intervals due to the loss or red blood cells and plasma in equal proportions. As time elapses, the PCV may initially remain withi (or even above) the laboratory reference range while the TP values progressively declines.
•	The discrepancy in PCV and TS is subsequent to: a) splenic contraction-induced autotransfusion fostering a normal to high PCV, in conjunction with b) transcapillary fluid shifts from the interstitial the intravascular space causing a dilutional effect on remaining serum protein concentrations.
•	Eventually, both PCV and TS values decrease as hemorrhage and subsequent fluid resuscitation ensues. Declining peripheral PCV/TP values with increasing progressive signs of circulatory shock an



Clinical and Diagnostic Considerations for Predicting Transfusion Needs

- In the context of trauma, suspect acute blood loss in any polytrauma patient that **presents with a** "low, normal or high" PCV and a low TS (≤ 4.5 g/dL).
- Declining peripheral PCV/TS values with increasing abdominal effusion PCV/TS is supportive of ongoing, active intra-abdominal hemorrhage.

Lactate and BE

- High serum lactate (particularly, 5 mmol/L or greater) in combination with lower PCV and TS concentration is consistent with the need for transfusion³:
- BE values -6.6 or lower is supportive of transfusion needs. In canines with blunt trauma, arterial BE may serve as a predictor of mortality and blood transfusion requirements within 24 hours of admission.¹²
- Lactate and BE can be performed on most blood gas analyzers; either arterial or venous blood can be used to determine these values.
- Studies have shown the presence of cryptogenic shock in patients with elevated base excess (BE) or serum lactate level in face of systolic blood pressures at appropriate values.¹⁵

Diagnostic Imaging

- Survey radiographs in conjunction with a TFAST and AFAST, when available, are recommended diagnostic modalities to include during the primary survey to increase the diagnostic sensitivity of identifying internal hemorrhage. Additionally if available, computed tomography can be utilized as a more sensitive modality to determine extent of traumatic injuries and identification of occult hemorrhage. Clinicians should be aware of the need to not only acquire images in this modality but the need for interpretation of the data that is generated.
- Abdominal fluid score(AFS) in MWDs with traumatic hemoperitoneum:
 - AFS of 1 or 2 are considered small-volume bleeders, these patients rarely become anemic from the intraabdominal hemorrhage.
 - AFS of 3 or 4 are considered large-volume bleeders and are more likely to become anemic and warrant transfusion therapy.¹⁶

Table 7: Canine Vital Signs - Perfusion parameters based on severity of circulatory shock

Stage of shock	HR*	CRT	ММ	Mentation	Pulse Quality	SBP
Normal (at rest)*	< 120	< 2 s	Pink	Bright, alert	Strong	> 90
Acute Compensatory	> 120	< 1 s	Red	Normal	Fair	> 90
Early Decompensatory	> 140	> 2 s	Pale	Depressed	Weak	< 90
Terminal	< 80	absent	Pale	Obtunded	Absent	Low

*Normal values are for a RESTING state. Exercise/activity, pain, stress will result in higher HRs



Table 8: Available blood products and indications for use

Product	Components	Indications		
Fresh Whole Blood (FWB) Use within 4 - 6 hours of collection	 Red Blood cells (RBCs) and White Blood Cells (WBCs) Plasma proteins^a All coagulation factors Platelets 	 Severe, acute hemorrhage Anemia^c + Hypovolemia Anemia + Coagulopathy / ATC / TIC Severe thrombocytopenia^d 		
Stored Whole Blood (SWB) Stored at 4°C for up to 28 days (depending on anticoagulant)	 RBC, WBCs Plasma proteins^a, coagulation factors, platelets (decreased circulation time) 	 Severe, acute hemorrhage Anemia + Hypovolemia Anemia + Coagulopathy / ATC / TIC 		
 Fresh Frozen Plasma (FFP) Frozen at < -18°C within 8 h from collection Good for 1 year 	 All coagulation factors Plasma proteins^a Antithrombin 	 Coagulopathy / ATC / TIC Colloidal volume resuscitation Hemorrhagic shock as bridge to whole blood replacement 		
 Freeze Dried Plasma (FDP) Lyophilized canine plasma Reconstituted with water and then stable for 28 days refrigerated 	Essentially bioequivalent to FFP	 Coagulopathy / ATC / TIC Colloid volume expander Hemorrhagic shock as a bridge to whole blood replacement 		
 ^a Plasma proteins include: albumin, globulin, and fibrinogen ^b Consider Acute Blood Loss/Hemorrhage indicated if one or more of the following: Estimated or anticipated blood loss > 20% of eTBV, about 400 – 500 mL in a 25 kg MWD. (eTBV in MWD = 85 mL/kg) Systemic signs of hypoperfusion. (See Table 1.) SBP < 90 mm Hg or drop in SBP by ≥ 30 mm Hg Hypothermia (< 99°F or < 37°C) Hyperlactatemia (≥ 5 mmoL/L) and / or Base excess -6.6 or lower 				
 ^c Symptomatic anemia resulting in: Tachycardia (HR ≥ 160 ± 20 beats/minute) ± bounding femoral pulses Pale to white mucous membranes Depressed mentation Presence of worsening anemia with signs of impaired oxygen delivery: 				



Table 9: Indications for blood products use by condition

Indications	Recommended Component (In Order of Preference)
AnemiaHypovolemiaConcurrent coagulopathy	Fresh or Stored Whole Blood
Severe, acute hemorrhage	 1st: Fresh or Stored Whole Blood 2nd: pRBCs + FFP or (FDP) (1:1 or 1:2 ratio) 3rd: FDP or FFP as resuscitative fluid until whole blood can be administered
 Thrombocytopenia (< 15,000) Presence of spontaneous hemorrhage Presence of petechial or ecchymosis 	1 st : Fresh whole blood 2 nd : Stored whole blood
Coagulopathy • Idiopathic / inherited ATC/TIC	1 st : Fresh Frozen Plasma or FDP 2 nd : Whole Blood (consider patient volume status)

WHOLE BLOOD TRANSFUSION

Whole blood (WB) is the preferred product for resuscitation of severe hemorrhagic shock in an MWD. Canine WB contains a biologically balanced ratio of all canine blood products and, as such, is the product most likely to restore effective circulating volume and oxygen carrying capacity while preventing coagulation alterations. Any MWD that requires a transfusion subsequent to trauma-induced hemorrhage should receive WB as the product of choice when available.

The primary blood group classification of clinical significance for transfusion in MWDs is based on the dog erythrocyte antigen (DEA) system. Although approximately 13 antigen specificities have been identified, only the DEA 1 system (particularly DEA 1.1) is of most concern due to its high degree of antigenicity and ability to result in an acute hemolytic transfusion reaction. Naturally occurring anti-DEA 1 alloantibodies associated with acute hemolysis (< 24 hours post-transfusion) are not present in canines; therefore, a cross match prior to a MWD's first transfusion is generally not necessary in a naïve recipient.¹⁷

Note: Transfusion of DEA 1-positive blood to a DEA 1-negative MWD will cause the recipient to develop antibodies against the DEA 1 positive antigen; this places the MWD at risk for future transfusion reactions.

To mitigate the risk of a potential transfusion reaction, the recipient's blood type and previous transfusion history should always be confirmed prior to any transfusion. A crossmatch compatibility should be done for any MWD that has received a previous transfusion ≥3 days prior to their next transfusion or if previous transfusion history cannot be confirmed. See below section on typing and cross matching.



HANDLING/STORAGE

- Fresh WB is collected from a donor and ideally administered to the recipient within 4-6 hours of collection.
- After 6-8 hours, the product becomes stored/chilled WB and should be kept at 4-6 degrees Celsius in a refrigeration device approved for chilled blood product storage.
- The shelf life of canine WB at 4-6 degrees Celsius depends on the anticoagulant used when the blood was collected.
- Canine WB should be stored refrigerated for up to 21 days when collected in CPD and up to 28 days when collected with CPDA-1. (Note: the 28 day shelf life of canine whole blood is different than the shelf life of human whole blood based on currently available in vitro data on refrigerated canine whole blood.²)
- WB should never be left at room temperature for longer than 4-6 hours and should never be frozen.
- When transporting canine WB, ensure that it is packaged in appropriate insulated containers on wet ice to maintain proper temperatures.

DOSAGE

- In most MWDs, a reasonable starting dose for a dogs needing a WB transfusion due to acute blood loss is one 450-500 mL bag (unit) of WB. Goal-directed clinical and laboratory reassessment of MWD should be done after the first bag of WB to determine if more blood is indicated.
 - A rough estimate of 2 ml/kg of WB is required to raise the patient PCV by 1%.
 - The desired target PCV to reach is one that results in sustained improvement of clinical signs and laboratory values for the MWD, usually >20-25%.
 - ^a Simply targeting a normal PCV range may not be necessary and could result in increased risk for undesirable transfusion reactions.
- Transfusions that take longer than 4-6 hours to complete are at risk for bacterial contamination and subsequent adverse effects.
- In a controlled setting involving a hemodynamically stable MWD, start with an initial WB transfusion rate of 0.5 to 1 mL/kg/h for the first 15 minutes and monitor for any adverse transfusion reactions. If no transfusion reactions are observed, increase the rate to provide the remaining amount within 4 hours; recommended WB transfusion rates for normovolemic canines is 5 – 10 mL/kg/h.
- In emergent situations involving severe, acute blood loss leading to trauma-induced hemorrhagic shock, a goal-directed WB transfusion rate of up to 20 mL/kg/h or 1.5 mL/kg/min over 20 minutes can be administered to restore hemodynamic stability.
- Reassess the MWD after the first unit of WB to determine if a subsequent transfusion needed.

ADMINISTRATION

- When possible, chilled WB should be passively warmed to room temperature over 30-60 minutes prior to administration, or passed through an IV infusion line warmer during administration. A gravity drip with an in-line adult human 170-260 micron filter set is recommended for the administration of canine WB to an MWD unless a blood specific infusion pump is available; WB should not be run through a standard fluid pump in order to preserve the structure of the donor RBCs.^{18,19}
- Administration to the MWD patient should occur through either a dedicated, large-bore (18 gauge or larger) peripheral or central intravenous catheter or an intraosseous catheter within a span of 4 hours. No other



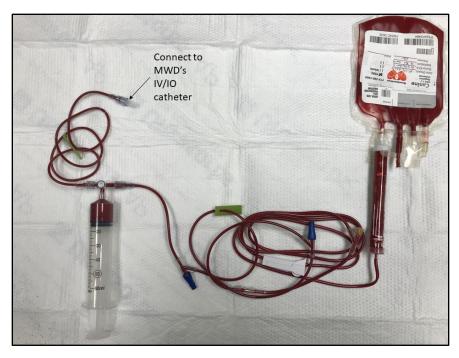
fluid therapy or medication administration should occur simultaneously through the same catheter supporting a WB transfusion.

- Administration of WB by gravity flow is recommended in canines. In order to preserve the integrity of donor RBCs,²⁰ it is not recommended to administer WB through an infusion pump unless the pump and administration set are designed and validated for use with blood or other blood component (pRBCs, plasma, albumin, platelets).
- Transfusions that take longer than 4-6 hours to complete are at risk for bacterial contamination and subsequent adverse effects.
- In emergent situations, canine WB can be transfused rapidly to maintain effective circulating volume, oxygen carrying capacity and to prevent cardiovascular collapse. This can be accomplished by one of the below methods:
 - 1. Manually squeezing the bag of whole blood or by placing the bag of whole blood in a pressure sleeve.

OR

- 2. Employ a rapid transfusion set (See Figure 1 below.)
 - a. Spike the blood bag with a standard blood administration set.
 - b. Connect a 3-way stopcock to the administration set and then connect a 60 mL syringe and an extension set to the 3 way stopcock.
 - c. Pull blood from the bag into the 60 mL syringe and prime the line and the extension set.
 - d. Ensure any air is removed from the circuit and connect the extension set to the MWD's catheter.
 - e. WB can then be rapidly pushed from the syringe to the MWD and refilled from the bag without disconnecting the set.

Figure 1. Example of WB set up for rapid infusion





Transfusion monitoring and reassessment

- 1. Measure and record baseline values of vital signs and perfusion parameters prior to the start of the transfusion. Values to measure include: rectal temperature, heart rate, pulse rate and quality, respiratory rate, mucous membrane color, capillary refill time, and blood pressure (if equipment is available).
- 2. Reassess vital signs:
 - a. Every 5 minutes for the first 15 minutes, then
 - b. Every 15 minutes for the first hour, then
 - c. Every 30-60 minutes until completion of the transfusion.
- 3. Capture a final set of vital signs at the conclusion of the transfusion.
- 4. Re-evaluate a PCV and TS one to two-hour post-transfusion.

Transfusion reactions

- Transfusion reactions of concern with canine WB transfusions include acute hemolysis, allergic/anaphylactic hypersensitivity, febrile non-hemolytic, and delayed hemolysis. (See Table 10 below.)
- Supportive treatment should address the type and severity of the reaction, but in all cases the transfusion should be stopped immediately to allow for further assessment of the patient and donor bag before continuing.
- For mild reactions, decreasing the transfusion rate (typically decrease the rate by 50%) may be all that is
 needed to avoid further issues. If signs of transfusion reaction persist or worsen, the transfusion should be
 halted and the supporting 64F should be consulted.

Note: A 64F is a veterinary clinical specialist typically found at the veterinary unit co-located with the human Role 3 and higher facilities. A 64F can also be reached via the ADVISOR Trauma Care Helpline, Option #5.

Transfusion Reaction	Clinical Signs
Acute Hemolytic Reaction Result of recipient immunological reaction to donor red cell antigens	 Fever Weakness, tremors Salivation, vomiting and/or elimination Agitation, vocalization Tachycardia, arrhythmias Tachypnea, dyspnea Hypotension Hemoglobinemia Hemoglobinuria
Delayed Hemolytic Reaction Result of recipient immunological reaction to donor cell antigens	 Clinically less severe Acute Hemolytic Reaction Occurs >2 days post-transfusion

Table 10. Transfusion reactions¹⁹



Transfusion Reaction	Clinical Signs	
Febrile, Non-Hemolytic Reaction Result of white cell antigens and other bioactive substances in donor blood	 Temperature increase of >1° Celsius (~2°Fahrenheiht) Vomiting Tremors Resolution in 1 to 12 hours 	
Hypersensitivity Transfusion Reaction Result of recipient IgE-mediated reactions to donor gamma globulins	 Mild to severe urticaria Mild to severe angioedema and erythema Anaphylaxis, bronchoconstriction and effusions in severe cases Typically no fever expected Can occur within minutes to hours of starting 	
Pre-Transfusion Hemolysis Result of improper storage or handling of donor blood, or bacterial contamination	Signs will mimic Acute Hemolytic Reaction	
Transfusion Related Circulatory Overload (TACO) Related to large volume and massive transfusions	 Hypocalcemia from citrate intoxication Tremors and seizures Arrhythmias Dilution thrombocytopenia or coagulopathy Hypomagnesemia Acid-base disturbances Hypothermia 	
Transfusion-Related Acute Lung Injury (TRALI) Rare in canines	 Tachypnea, tachycardia and respiratory distress related to development of non-cardiogenic pulmonary edema 	

FRESH FROZEN PLASMA TRANSFUSIONS

Canine FFP is canine WB that is separated from its cellular components and frozen within 8 hours of collection. FFP can be stored frozen for up to one year which then is relabeled as frozen plasma (FP). FFP contains all coagulation factors and plasma proteins found in circulating blood. FP contains most of the coagulation factors but loses the labile factors (FV, FVIII, von Willebrand factor) over time.

USES

- FFP is indicated to correct coagulation factor deficiencies and or prophylactically to treat MWDs who are at risk of bleeding from clotting factor abnormalities.
- As compared to isotonic crystalloids, canine FFP provides the following added benefits as a resuscitation fluid:



- Supports hemostatic resuscitation. Its ability to deliver coagulation factors and other plasma proteins (e.g. albumin, fibrinogen) while restoring intravascular volume minimizes the risk of invoking a dilutional coagulopathy.
- Provides oncotic support by supplementing albumin in addition to restoring effective circulating volume.

AVAILABILITY

- FFP comes in different sized units but 240 to 250 mL units are most appropriate for MWDs.
- FFP is obtainable from one of two methods:
 - **1.** Separation of Fresh Whole Blood (FWB):
 - Place a unit (450 to 500 mL) of FWB into a blood centrifuge and spin at 2000 x g for 10 minutes to separate the cellular components.
 - Express the plasma from the primary collection bag into a satellite bag and then seal.
 - Place plasma in freezer at -20°C to -80°C (-4°F to -112°F) within 8 hours of collection if it is intended to serve as FFP.
 - **2.** Plasmapheresis is an alternate way to obtain FFP; however, this process requires special training and equipment. Only trained personnel with the appropriate equipment should perform plasmapheresis.

HANDLING/STORAGE

- FFP should be stored in a dedicated, appropriate freezer at -20 to -80°C. Perform daily temperature checks to ensure the freezer is secured and operating appropriately.
- If FFP is to be shipped it should be shipped with proper insulated packaging on dry ice to ensure the product remains frozen at the correct temperatures.

Note: Care should be taken when handling FFP as the plasma bag is not pliable and can crack at low temperatures. Consider placing in two large Ziploc[®] bags or similar packaging to avoid direct handling of the FFP bag.

DOSAGE AND ADMINISTRATION

- Typical dose of canine FFP is 10 to 20 mL/kg over 1- 4 hours (depending on the situation). This translates to approximately one to two 240 to 250 mL bags of FFP per MWD.
- FFP should NOT be used to try to increase plasma albumin levels as up to 40 to 50 mL/kg could be necessary to raise recipient's albumin levels by 1.0 g/dL.
- MWDs that do not have ongoing hemorrhage or other substantial fluid losses, administration of FFP over 2-4 hours is appropriate.
- FFP must first be thawed before administration.
 - Optimally, thaw FFP in a commercial plasma thawer following manufacturer's recommendations for thawing human plasma.
 - If no plasma thawer is available, remove FFP from the freezer and place in two Ziploc[®] bags. Submerge the FFP and outer Ziploc[®] bags in lukewarm tap water. A large MWD food bowl is often suitable for this purpose.

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- Ensure the water does not exceed 98.6°F (37°C); water temperatures exceeding 98.6°F (37°C) may cause protein denaturation. It is important that the thawing process does not occur too quickly by adding hot water as plasma proteins can be denatured if the process is rushed. Consider exchanging the water every 15 to 20 minutes or use a gentle continuous flow of water if necessary to keep the water bath warm. Expect 30-40 minutes to complete the thawing process using this technique.
- Once thawed, spike FFP bag with a blood administration set (which contains a 170 micron blood filter) or a regular fluid administration set. If no blood administration set is available, place a blood filter (Hemo-Nate[®] filter) in the transfusion line to reduce transfusion of clots or other impurities from the transfused product. Do not let the lack of a Hemo-Nate[®] (or similar blood filter) preclude the administration of FFP if it is clinically necessary.
 - 1. Connect the primed transfusion line to a 20 gauge or larger bore peripheral or central IV catheter or an IO catheter.
 - Begin transfusion at 1 to 2 mL/kg/h over the first 15 minutes and monitor the MWD for signs of transfusion reaction. (See <u>Table 10</u>.) If there is no significant reaction, increase the rate of transfusion to 2 to 4 mL/kg/h for an additional 15 minutes. If no transfusion reactions are seen over the first 30 minutes, increase the transfusion rate to ensure the complete delivery of FFP within 2 to 4 hours.
- In emergent situations, consider blousing 1-3 units thawed FFP to MWDs similar to that indicated for MWDs that are rapidly losing blood form a major blood vessel laceration. Bolus the whole unit of FFP over 10 minutes by one of the following techniques:
 - Placing the FFP bag in a commercial IV pressure infuser system or manual squeezing of the FFP bag, OR
 - ^o Using the 3-way stopcock and 60 mL syringe set-up described in the WB administration section above.

Transfusion monitoring and reassessment

- Vigilant monitoring for transfusion reactions is required during non-emergent FFP transfusions (e.g. in absence of bolus FFP therapy). Types and signs associated with transfusion reactions are similar to those for WB transfusions. (See above section on transfusion reactions for WB.)
- Monitor core body temperature, pulse rate and quality, respiration (rate and effort) and blood pressure:
 - Every 5 minutes for the first 15 minutes of transfusion, then
 - Every 15 minutes for the first hour, then
 - Every 30-60 minutes thereafter until completion of the transfusion.
- The most common indications of a transfusion reaction are fever, facial edema, tachycardia, tachypnea, vomiting, hypotension and weakness.⁷
 - If any of these signs are encountered, reduce the transfusion rate by 50% and reassess the MWD. If clinical signs resolve, continue the transfusion.
 - If signs of transfusion reaction persist or worsen despite reducing the transfusion rate, halt the transfusion and seek consultation with the supporting 64F.
- Reassess the MWD upon completion of the transfusion. Assessment includes a physical examination with special attention to the cardiovascular, coagulation and perfusion parameters to include blood pressure assessment. If end points of resuscitation are not reached, consider administering an additional unit of FFP following the guidelines above.



Continue hemorrhagic shock resuscitation with FDP or FFP until canine WB is available to administer.

FREEZE DRIED PLASMA

Canine Freeze Dried Plasma (cFDP) can be used in lieu of FFP for any of the above-mentioned indications cited for FFP. In addition, since cFDP can be reconstituted quickly (does not require a thawing process), it is possible to start a transfusion more rapidly as compared to FFP. When available, strongly consider cFDP as a resuscitation product at the point of injury, en route to Role 2 or 3 care and/or until canine WB becomes available. cFDP replaces effective circulating volume without diluting coagulation factors or substantially reducing oncotic pressure. In this way, cFDP's most beneficial role on the battlefield is to serve as bridge to resuscitation with canine WB. Evidence supports that cFDP is biologically equivalent to FFP in terms of coagulation factor activity, biochemical profile and clot forming ability (Edwards – unpublished data).

HANDLING/STORAGE

- cFDP is available as a white to yellow powdered product within a transfusion bag that is enclosed in a Mylar outer wrap. To minimize any transfer of moisture from the environment into the product, do not open the Mylar wrap until the product is ready for use.
- Store cFDP at room temperature or refrigerated at 2 to 6°C (35.6 to 39.2°F).

RECONSTITUTION

Follow the manufacturer's directions in the reconstitution of cFDP. In general, sterile water for injection (amount specified by the manufacturer) is aseptically added through either an inserted port or the designated port provided on the bag of cFDP. Gently manually mix the bag until the product completely dissolves (approximately 5 minutes). Once reconstituted, either transfuse within 4 hours of reconstitution or refrigerate for up to 28 days.

DOSAGE

- MWDs in need of a plasma transfusion should typically receive one 240 to 250 mL bag (unit) of cFDP; reassess after the first unit to determine if the MWD requires subsequent transfusions.
- The speed of administration depends on the indication and clinical condition of the MWD.
 - For MWDs that do not have ongoing hemorrhage or other substantial fluid losses, administration of FDP over 2 to 4 hours is typically appropriate.
 - In emergent situations, bolus FDP as indicated for MWDs that are rapidly losing blood. Boluses of 1-3 units may be necessary to stabilize a severely injured MWD. To accomplish this, a unit of FDP may be placed in a commercial pressure infuser sleeve or can be accomplished by using the 3 way stopcock and 60 mL syringe set up described in the WB administration section above.



ADMINISTRATION

- If the bag of FDP has an inline filter, attach extension sets to the inline filter port and prime the line with FDP to remove the air. If no inline filter is present, spike the bag of FDP with either a:
 - Blood administration set (that contains a blood filter), OR
 - Regular fluid administration set that has a separate blood filter (such as a Hemo-Nate[®] filter) placed in the transfusion line.

Note: Although an in-line filter is recommended to reduce clots or other impurities from the transfused product, the lack of a blood filter does not preclude the administration of FDP if the transfusion is clinically necessary.

- Connect the primed transfusion line to a 20-gauge or larger peripheral or central IV catheter or an IO catheter.
- Begin transfusion at 1 to 2 mL/kg/h over the first 15 minutes and monitor for signs of transfusion reaction (see below). If no significant reaction observed, increased the rate of transfusion to 2 to 4 mL/kg/h for an additional 15 minutes. If no transfusion reactions observed over the first 30 minutes, increase the transfusion rate to ensure delivery of the FDP unit is complete within 2 to 4 hours.

TRANSFUSION MONITORING AND REASSESSMENT

Transfusion monitoring and assessment in the administration of FDP is the same as for FFP detailed above.

BLOOD DONATIONS (WALKING BLOOD BANKS)

When canine WB is not available and is needed for a critically injured or ill MWD, blood can be collected from other canines for donation. U.S.-based MWDs are the recommended first-choice for walking-blood donors, since the U.S. Army Veterinary Corps knows that these canines are well-cared for, healthy, up-to-date on all major vaccinations and preventative medications, and clear of blood-borne infectious diseases. If a U.S.-based MWD is not available, consider a US based contract working dog as an alternative donor. Due to the high risk of disease transmission, NEVER use an indigenous canine as a blood donor. An ideal MWD to select for further screening is a canine that has never received a prior transfusion of any blood product (to include plasma products), weighs over 60 pounds (27 kg), are deemed healthy (based on physical examination), and not receiving any medication other than standard preventive medications (heartworm, flea and tick).

SCREENING²¹

- If not previously known or performed, use a commercial DEA-1 blood-type kit to determine the blood type of the potential MWDs (see below section on typing and cross matching).
- Screen ALL canines for infectious diseases caused by Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis, Ehrlichia ewingii, Anaplasma phagocytophilum, Anaplasma platys, Leishmania utilizing in-house diagnostic tests.
- Obtain a minimum database (complete blood count, serum chemistry profile, urinalysis, and fecal floatation) to assure that no underlying conditions are present that precludes donation.



 MWDs that are negative for infectious diseases and have no significant abnormalities noted on their minimum database are considered acceptable donors unless other extenuating circumstances deem the donor not acceptable.

Note: In an emergent situation when time is critical, it is reasonable to consider any healthy MWD as a blood donor even if they have not had the aforementioned screenings.

BLOOD COLLECTION

- Do not collect blood from donors more frequently than every two months.
- Do not work or fly (via fixed wing aircraft) a donor for 24 hours following a donation to allow recovery from donation.
- Consider administering intravenous isotonic crystalloids to a donor following blood collection as needed to replace the volume donated.

Anticoagulants.

Recommend the use of CPDA-1 (citrate phosphate dextrose adenine). CPDA-1 results in the longest blood shelf life at 28 days for canine WB. If CPDA-1 is not available, collect WB into bags containing CPD.

Note: Canine WB anticoagulated with CPD results in a recommended shelf-life of up to 21 days when properly stored at 6°C (+/- 2°C). Approximately 1 mL of anticoagulant is used per 9 mL of blood collected from a dog. **Caution**: **Accidental intravenous injection of undiluted citrate anticoagulants may cause cardiac arrest.**

Sedation

- Always take the MWD's temperament into consideration prior to administering any sedatives. Although
 most MWDs require sedation in order to keep them still to facilitate blood collection, not all MWDs require
 the same levels of sedation.
- For most MWDs, consider the following initial protocol:
 - Butorphanol (0.1 0.4 mg/kg IV/IM) combined with,
 - Midazolam (0.25 0.5 mg/kg IV/IM).

If butorphanol is not available consider hydromorphone (0.05-0.1 mg/kg) IV/IM combined with Midazolam

Considering the average size MWD weighs approximately 30 kg, consider the following doses as acceptable for initial sedation in situations when a MWD's weight is unknown and/or not obtainable:

- Butorphanol (3 to 12 mg IV/IM) OR hydromorphone 1.5-3 mg IV/IM combined with,
- Midazolam (8 to 15 mg IV/IM).

Note: Most MWD Handlers have a weight-based MWD drug card that includes drugs doses that are specific to their MWD. Check for this card prior to making any weight-based drug calculations.

- Some MWDs may require heavier sedation to facilitate blood collection and avoid complications such as damage to the blood vessel (aka. blown vein) leading to contamination and/or insufficient collection. For these cases, consider adding ketamine (2 to 4 mg/kg IV/IM) to the above listed protocol.
- AVOID the following drugs in the sedation protocol:



- 1. Acepromazine: May influence platelet function; therefore, is not recommended in situations that warrants active platelets.
- 2. Dexmedetomidine: May causes clinically significant bradycardia and peripheral vasoconstriction, which interferes/hinders blood flow and collection.

Procedure

- Employ aseptic technique
 - 1. Wash hands with soap and water prior to the procedure and don sterile gloves.
 - 2. Clip and surgically prepare the area over the external jugular vein.
- Do not allow air to enter the blood collections system. Most commercial blood collections systems designed for humans possess an in-line device that prevents air from entering the line. If not using a human-designed commercial blood collection system, consider the following to prevent air from entering the system.
 - 1. Clear the collection line of air by turning the bag upside down, removing the needle cover and allowing a few drops of anticoagulant to exit the needle.
 - 2. The collection line is then immediately clamped and not released until the venipuncture has been accomplished.
- Using a standard human tri or quad commercial blood donation bag set, sterilely insert the attached needle into the left or right external jugular vein. Once inserted, the in-line cannula is broken to allow the flow of blood to begin.
- Utilize a balance or gram scale for collection. Prior to collection, tare the scale with the collection bag to "Zero".
- Blood is collected by gravity flow into a standard 500 mL CPD or CPDA-1 blood bag to the appropriate volume.
 - 1. Place the MWD in lateral or sternal recumbency. If the MWD is in lateral recumbency, place a towel underneath the neck in order to facilitate obtaining and preserve venipuncture of the jugular vein.
 - 2. Place the collection bag on the floor or at a location lower than the MWD.
- Ensure the ratio of blood collected to anticoagulant is exact to assure an appropriate level of anticoagulation as well as to maximize cell viability:

The specific ratio is 450 mL blood +/- 10% (i.e. 405 mL to 495 mL) per 63 mL of anticoagulant (CPD or CPDA-1).

- Gently rock the bag during collection to insure adequate mixing of the blood and anticoagulant.
- Discontinue collection when the total weight of the blood collected is 430 to 450 grams.
- At the end of collection:
 - 1. Clamp or tie off the line before removing the needle from the vein; this prevents air from entering the bag before all the blood has cleared the walls of the line.
 - 2. Remove the needle from the vein and apply appropriate pressure to the venipuncture site for at least 5 minutes to prevent further bleeding.



- After the collection line is tied or clamped, use a tube stripper to "strip" the blood left in the line into the bag.
 - 1. Gently rotate the bag to mix the blood and anticoagulant;
 - 2. Invert the bag and allow the line to refill.
 - 3. Repeat four to five times to assure thorough mixing of the blood in the line with the anticoagulant in the bag.
- When collecting WB, satellite bags should be removed using heat sealers or clamps leaving only the collection bag containing WB.
 - 1. The line is then heat sealed, clamped or tied in segments for use in future cross-matches.

Note: The line has a series of numbers printed on the surface that correspond to segments. Fill the line, then use the heat sealer to separate it into appropriate segments (approximately 6 to 8 segments) or 'pigtails'. These "pigtails" are used for cross-matching.

- 2. Heat seal the line then cut the needle from line and discard the needle according to safety procedures.
- Mark the outside of the bags with: collection date, donor's name, weight of the bag, and PCV of the donor. Make three labels – one for the transfusion sheet, one to remove and place in the inventory log book, and one to stay on the bag.
- Store fresh WB upright in a designated refrigerator, with a temperature record maintained daily and recorded on the outside of the refrigerator.

Table 11. Supplies needed for MWD blood donation

Donor Screening Supplies	Sedation Supplies (If Needed)	Collection Supplies
 Syringes, needles, and tubes for blood and urine collection Leishmania tests –(if in endemic area) (NSN 6640-NCM093803) Idexx SNAP 4Dx Plus tests (NSN 6550-016504412) Canine blood typing cards - Rapid Vet (NSN 6550-016479167) CBC, Chemistry, UA, and Fecalyzer 	 Atropine sulfate Butorphanol +/- Midazolam +/- ketamine Syringes, needles, calculator 	 4x4 gauze sponges Sterile scissors Sterile draping cloth Sterile gloves Whole Blood Collection Bags (CPD or CPDA-1) Balance or gram scale Strippers Metal clamps or heat sealer
Supportive Care Supplies	Aseptic Prep Supplies	Crossmatch Supplies (Optional)
 18 G IV Catheter supplies Crystalloid fluids 1 liter (x 2) Primary IV set Pressure infusion bag or fluid pump Vitals Monitor 	 Clippers Rolled towel to support neck Exam Gloves Chlorhexidine or betadine surgical scrub Sterile gauze Sterile saline or Alcohol 	 0.9% saline 3 mL test tubes Pipettes Centrifuge Agglutination viewer lamp (optional, microscope also works)



TYPING/CROSSMATCHING OF MWDS

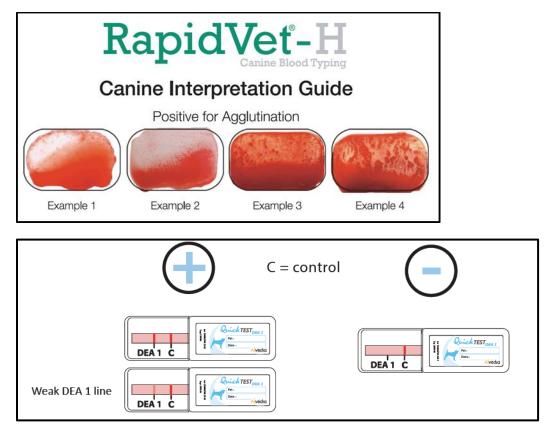
MWDs or other CWDs operating in a deployed environment may or may not have their blood type identified prior to deployment. Identification of a canine's blood type during deployment and prior to transfusion is always recommended but might not be possible unless commercially available blood typing kits (See Figure 2.) are fielded at a Role 2 or Role 3 Veterinary Facilities. For reasons explained below, naïve canines that have never received a prior blood product transfusion can safely receive blood transfusions for a short period of time prior to both typing and cross-matching, if necessary.

The most commonly tested and reported blood type in canines is DEA 1. In addition to DEA 1, there are 12 other DEA blood types that are based on antigenic surface markers of the canine erythrocyte.²² Generally speaking, only the presence of the DEA 1 antigen has historically resulted in clinically relevant transfusion reactions in dogs. As a result, most commercially available blood typing kits screen specifically for the presence (DEA 1 positive) or absence (DEA 1 negative) of the DEA 1 antigen. MWDs and other operational canines will therefore have a blood type reported as DEA 1(or with the older naming convention DEA 1.1) positive or negative if they have been previously blood typed. Although DEAs could theoretically cause transfusion reactions, there is no evidence in the veterinary literature to support routine screening of these antigens.

It is important to note that canines do not typically have naturally circulating alloantibodies to the DEA 1 antigen. Therefore, it is possible to transfuse non-typed canine blood products into another MWD and not experience a transfusion reaction – giving rise to the adage that "the first transfusion is free." In practice, the veterinary personnel and other HCPs in theater can emergently provide blood products to MWDs without knowing the blood type of the MWD during the first 72 to 96 hours of care. However, dogs will develop alloantibodies to the DEA antigens 5 to 7 days post-transfusion and should be cross-matched 72 hours after receiving the first transfusion.

Popular, commercially available blood typing kits include point-of-care kits made by RapidVet[®] and Alvedia (<u>Figure 2</u>). Both kits are simple, inexpensive, and easy-to-use. However, the user should be advised that erythrocyte auto-agglutination can affect interpretation of the RapidVet[®] test kit. If erythrocyte agglutination is present, a cell washing step is required prior to using the typing cards. The Alvedia test kit is unaffected by cell agglutination due to its patented membrane technology embedded in the testing cartridges.

Figure 2. Examples of Common Blood Typing Kits.



All MWDs should be cross-matched if 72 hours have elapsed since its first transfusion or if any previous transfusion resulted in a transfusion reaction. Crossmatching is the process by which donor and recipient blood are assessed for compatibility and thus reduces the likelihood of a transfusion reaction.

There are two types of cross-matching: Major and Minor.

- 1. **Major Crossmatch:** Recipient's serum is mixed with a suspension of the donor's red blood cells. A major crossmatch detects antibodies present in the recipient's serum that will react to antigens present on the donor red blood cells.
- 2. **Minor Crossmatch**: Recipient's red blood cells are mixed into the donor's serum. A minor crossmatch detects antibodies present in the donor's serum that will react to antigens present on the recipient's red blood cells.

Performing a cross-match is accomplished using either a/an:

1. Commercial cross-matching test kit:

RapidVet[®] provides a testing kit called the RapidVet[®]-H Companion Animal Crossmatch Test for both major and minor crossmatching. The procedure involved in this kit is very similar to traditional laboratory based crossmatching and personnel should refer to manufacturer's directions for the proper employment of the test kit. The advantage in using the RapidVet[®] kits is that the gel-impregnated kits



used to do the final assessment prevent artifact if naturally occurring red blood cell auto-agglutination is present.

- 2. In-house, field-expedient procedure described below in Table 12. Equipment required:
 - Small centrifuge
 - Image result for Ethylenediaminetetraacetic acid (EDTA)
 - EDTA-impregnated blood collection tubes (purple top)
 - Non-serum separator blood collection tubes (red top)

Table 12. Field-Expedient Crossmatch Procedure.²³

Field-Expedient Crossmatch Procedure

- 1 Collect EDTA (purple top) blood samples from both the donor and recipient dog.
- 2 Centrifuge at 5000 x G for 5 minutes
- 3 Separate plasma from the purple top tubes and decant into red top tubes labeled "plasma."
- 4 Place remaining suspension of RBCs in red top tubes labeled "RBCs."
- 5 Fill RBC tubes with 2.5 mL sterile saline. Mix gently and centrifuge for 1 min @ 5000 x G.
- 6 Decant saline and repeat process 3 times to wash the cells of proteinaceous debris.
- 7 Resuspend RBC pellet with 2.5 mL of saline.
- 8 **Major Crossmatch**: 2 drops of plasma from recipient placed into a new red top tube with 1 drop of RBC solution from the donor.
- 9 **Minor Crossmatch**: 2 drops of plasma from donor dog placed into new red top tube with 1 drop of RBC solution from recipient.
- 10 **Recipient Control**: 2 drops of recipient plasma with 1 drop of recipient RBC solution in new red top tube.
- 11 All solutions are mixed gently and incubated for 20 min @ 37C.
- 12 Centrifuge solutions for 15 seconds.
- 13 Inspect tubes for macroscopic appearance of cell agglutination. Presence in either of the crossmatch solutions indicates incompatibility. Presence in the control tube indicates auto-agglutination and voids any further interpretation of cross-matching results with this procedure.
- 14 Place one drop on a slide with coverslip and inspect for microagglutination. If present, add a single drop of saline to confirm agglutination. Score agglutination as follows:
 - 4+ = Single solid clump of cells
 - 3+ = Several solid clumps of cells
 - 2+ = Multiple small clumps of cells with cell/debris-free background
 - 1+ = Hemolysis with no visible agglutination.
 - NEG = No hemolysis or agglutination present.
- 15 Blood is deemed fully compatible with NEG results. However, in practice 1+ agglutination score is generally compatible and safe for a transfusion.

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